Emergence and Spread of Antimicrobial-Resistant Pathogens in an Era of Globalization

Guest Editors: Abiola C. Senok, Giuseppe A. Botta, and Olusegun O. Soge
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In recent years, we have witnessed an increased emergence of antimicrobial-resistant (AMR) pathogens. In this era of globalization, international travel has been implicated as a significant risk factor for the acquisition of infections with multidrug-resistant bacteria, including *Acinetobacter* spp., methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, hypervirulent *Clostridium difficile*, and Extended-Spectrum-Beta-Lactamase- (ESBL-) producing Enterobacteriaceae [1–4]. The plasmid encoded cefotaximase enzymes CTX-M and the New Delhi metallo-beta-lactamase-1 (NDM-1) represent two excellent examples of the ESBL and carbapenemase that have been rapidly and globally disseminated. In this special issue, M. Elouennass et al. report on high rates of ESBL-producing Enterobacteriaceae and the emergence of carbapenemase-producing isolates in Morocco. The boom in medical tourism has seen patients from developed nations taking up low-cost private medical care in developing countries. This inevitably provides ample opportunity for clinically important AMR pathogens to be acquired and disseminated across geographical borders. A recent example is NDM-1 which was first described in an isolate from a Swedish patient who had previously been hospitalized in New Delhi, India [5]. NDM-1-producing isolates have subsequently been reported across several continents often detected in patients with history of recent medical care in the Indian subcontinent [6]. Preventive measures against nosocomial transmission of AMR pathogens include hand hygiene, environmental decontamination as well as screening and cohorting of patients. Data from the study by J. C. Catano et al., presented in this issue, identifies the diversity of areas of bacterial contamination in a tertiary healthcare setting in a developing country. As stated by the authors “these bacterial reservoirs are a plausible source of infections for patients” and it indicates the need for further research to evaluate strategies for minimizing risk of transmission to patients.

In recognition of the global threat of the emergence and spread of multidrug-resistant bacteria worldwide, the World Health Organization (WHO) selected combating antimicrobial resistance as the theme for World Health Day 2011, issuing an international call for concerted effort geared towards halting the spread of antimicrobial resistance and recommending a six-point policy package for governments [7]. The fourth policy statement is to “regulate and promote rational use of medicines, including in animal husbandry, and ensure proper patient care.” Antimicrobial stewardship interventions promote judicious use of antibiotics and are critical in reducing emergence of resistance. In this issue, S. J. Patel et al. describe the development of an audit and feedback intervention based on the principles of the “model of actionable feedback” as well as the challenges to its implementation in a Neonatal Intensive Care Unit.

In a seminal review in science in January 2000, Daszak, Cunningham and Hyatt underscored the “interaction with zoonotic pathogens within a host-parasite continuum between wildlife, domestic animals and human population”
[8]. What we contend here is that as microorganisms can move freely in the diverse environments, so therefore can antibiotic resistance move without borders among humans, animals, and plants. The challenge posed by the emergence/reemergence of pathogenic microorganism from such interplay between humans, animals, and the environment is so significant that in 2009 the Royal Society issued a statement (RS Policy Document 2/09) pleading a “holistic approach” to infectious diseases in order to reach a “greater synergy between human and animal health sectors” [9]. There is widespread use of antibiotics such as glycopeptides and fluoroquinolones in animal husbandry with majority of these (estimates up to 90%) being administered not to treat infections but more controversially as growth enhancers. Section 4d of the aforementioned WHO policy statement specifically calls for reduction of use of antimicrobials in food-producing animals [7]. In 2003 The Lancet hosted an exceptionally appealing forum on “People, animals and antibiotic resistance” in which Dr. Wegener of the Danish veterinary institute reported on the ban on avoparcin (a glycopeptide), virginiamycin (streptogramin) tylosin, and spiramycin (macrolides) as growth promoters in the European Union [10]. However, as the use of antibiotics in food animals and in agriculture continues in other jurisdictions, the potential for clinically relevant pathogens gaining resistance markers from resistant strains selected in the environment remains a real threat. Indeed last year, an international research team reported that strains of Enterococcus faecalis isolated from pigs corresponded to a clone disseminated in several hospitals in Italy, Spain, and Portugal, harboring the same indistinguishable 100 kb mosaic plasmid—perhaps unequivocal evidence of trafficking of resistance genes in the environment between food animals and humans [11]. Of greater concern is that these genes (whether they originated before or after the EU ban) are now firmly established in the healthcare setting. The mechanisms, patterns, and clinical implications of emergence of resistance to glycopeptides and fluoroquinolones are presented in two in-depth reviews in this issue. Antibiotics from these two classes have been used extensively in animal husbandry, and findings from these reviews show that the WHO call is timely.

The WHO recommends strengthening surveillance and laboratory capacity as the second policy statement for combating antimicrobial resistance [7]. The significant role played by international travel in the spread of multidrug-resistant bacteria urgently calls for a rapid and robust detection of these clinically important bacteria through functional surveillance systems. Most reports of importation of multidrug-resistant clinically important bacteria by returning travelers are from the industrialized countries with well-equipped clinical microbiology laboratory for rapid detection of new resistance mechanisms. Sadly, however, most developing countries where the multidrug-resistant bacteria successfully emerge and spread do not have functional clinical microbiology laboratories and the capacity for detecting bacteria with the novel resistance mechanisms. Disturbingly, developing countries are still plagued by inappropriate sewage systems, poor healthcare services, and severe overcrowding which together with unregulated use of antimicrobial agents favor the emergence and spread of multidrug-resistant bacteria. In this issue, P. Bhattar et al., provide us with a snapshot of the challenges to the public health system of two emerging economies (China and India) as they face the realities of tuberculosis control. The lessons learnt may serve as models for other developing countries. As recommended by the WHO, there is an urgent need for investment in global antimicrobial resistance surveillance systems, especially in developing countries to ensure prompt detection of emerging multidrug-resistant bacteria with novel clinically important resistance before their worldwide dissemination.

Another important concern that should deserve more attention is the problem of global spread of antifungal resistance. Indeed, no paper related to antifungal resistance was received for peer review for this issue. As noticeably stated in a recent editorial in Science, billions of patients are suffering from fungal infections, caused by over 600 different fungal species [12]. Those previously considered not clinically relevant like Fusarium, Malassezia, Paecilomyces, and Penicillium marneffei are emerging as important pathogens associated with significant morbidity and mortality. No vaccine is available to fight these infections, diagnostic tests are cumbersome, and rapid tests, such as the recent β-1-3D glucan assay or galactomannan, are lacking specificity and are not affordable in many laboratories. Delay in the diagnosis and initiation of treatment of fungal infection can have devastating consequences. The available drugs, such as Amphotericin B, are extremely toxic (or the less toxic formulations are exceedingly expensive); the azoles show pharmacokinetic, pharmacodynamics, and resistance problems, thus resulting in increasing misuse of the echinocandins in empirical therapy. There is some evidence that at least in Aspergillus fumigatus the observed azole resistance might be related to the widespread use of fungicide in the environment especially in agriculture where azoles (such as fenbucnavazole and propiconazole) are commonly used for plant protection. It is evident that the fact that molecules used in agriculture are different from those used in humans is in respect to the problem of emergence of resistance totally irrelevant. Extremely well-documented molecular investigations were presented by Verweij et al. [13], The authors found that the Aspergillus fumigatus multi azole resistance which emerged in the Netherlands since 1999, with 6–13% of patients harboring the isolate, became resistant through a single resistance molecular mechanism represented by a substitution at codon 98 of cytP31A and a 34 bp tandem repeat in the gene promoter region. This mutation was detected in 99% of isolates and strains found in the soil and compost were genetically related. The bad news is that reports of this mutation TR/L98H have emerged from other European Countries (Belgium, France, Norway, Spain, and the UK) indicating spread of the resistance trait. Future implications with tremendous economic impact of this discovery are extremely important for the public health. Most likely, as done for antibiotics in food animals, regulations will be needed to restrict the use of antifungal agents of selected classes in agriculture, although preventing the spread of
the current resistance might well prove to be a mission impossible.

Acknowledgment

We would like to thank Dr. Alessandra Arseze, University of Udine, Italy, for her kind assistance and input in the preparation of this special issue. The authors have no conflict of interests to declare.

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References


Review Article
Global Fluoroquinolone Resistance Epidemiology and Implications for Clinical Use

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Received 25 March 2012; Accepted 26 June 2012

Academic Editor: Abiola C. Senok

This paper summarizes data from local, national, international, and global surveillance studies of antimicrobial resistance combining the complementary approaches of

1. Introduction

Nalidixic acid—a byproduct of chloroquine synthesis—was marketed during the 1960s for oral treatment of urinary tract infections and is still available by prescription. Several quinolones were invented since then, including flumequine bearing a fluorine atom at position C-6, which was active against nalidixic acid resistant Enterobacteriaceae. However, development of newer fluoroquinolones did not progress significantly till it was demonstrated that substitutions at the C-6 and C-7 positions improved antibacterial activity and pharmacological properties [1]. Since then, fluoroquinolones have become established for treatment of urinary, respiratory, gastrointestinal, urogenital, intra-abdominal, and skin/skin structure infections in outpatients and hospitalised patients. Despite millions of prescriptions in the first two decades of their use, the emergence of quinolone resistance during treatment was uncommon except in Staphylococcus aureus particularly in methicillin-resistant S. aureus and P. aeruginosa. Resistance to fluoroquinolones emerged rapidly in these two species, predominantly due to clonal spread among nursing home residents and immunocompromised patients [2]. However, since the mid 1990s quinolone resistance started to increase in almost all Gram-positive and Gram-negative species and minimal concentrations (MICs) inhibiting 90% of the strains studied varied species specifically over a broad range from $\leq 0.015$ up to $\geq 128$ mg/L [3–5] thus indicating that resistant subpopulations were frequent already two decades ago but passed almost unnoticed. Recent surveillance studies demonstrate that resistance rates continue to increase thus affecting patient management and necessitating a change in current treatment guidelines [6, 7], or even precluding the use of fluoroquinolones in certain indications as will be discussed below [8, 9].

This paper summarizes data from local, national, international, and global surveillance studies of antimicrobial resistance combining the complementary approaches of
routine surveillance (the active investigation of results generated in the course of routine clinical care) and targeted surveys (one-time or periodic study protocols to address specific scientific or public policy needs not adequately addressed by routine diagnostic test results). Data generated in the course of global, longitudinal surveillance studies are complemented with national and regional data. Only those studies using standardized test methods and defined susceptibility/resistance-criteria according to national—or preferably CLSI—(formerly NCCLS) breakpoint definitions were selected. Many articles quoted in this paper originate from the author’s files; others were chosen from searches on PubMed. Articles summarized in recent reviews were excluded from this synopsis.

Large global surveillance studies comprising centers in Asia, Asia/Pacific region, Japan, North, Central, and South America, and the EU have the strength that large numbers of pathogens are sampled and that standardized methods of data collection, susceptibility testing and data interpretation are used. Therefore, surveillance programmes like SENTRY (a global, longitudinal study on the susceptibility of pathogens causing blood-stream infections, community-and hospital acquired RTIs, skin and soft tissues, and UTIs, sponsored by Bristol Meyers Squibb, recently switched to a study on the susceptibility of Gram-positive pathogens to daptomycin and comparators), MYSTIC (meropenem yearly susceptibility test information collection, a global, longitudinal surveillance study designed to evaluate the prevalence and in-vitro antimicrobial susceptibility of isolates from intensive care units, neutropenia units, cystic fibrosis units, or non-specialist centres where meropenem is used, sponsored by Astra Zeneca), SMART (study monitoring antimicrobial resistance trends, a study on the susceptibility of intraabdominal aerobic and anaerobic clinical isolates, sponsored by Merck), PROTEKT (prospective resistant organism tracking and epidemiology for the ketolide telithromycin, sponsored by Aventis Pharmaceuticals), GLOBAL (global landscape on the bacterial activity of levofloxacin) and the “Alexander Project” (an international study that began in 1992 and involved initially 6, later 27 countries, sponsored by GlaxoSmith Kline), data from major European programmes (e.g., European Antimicrobial Resistance Surveillance System (EARSS); ECO. SENS (E. coli sensitivity)) and national programmes (e.g., NAUTICA (North American Urinary Tract Infection Collaborative Alliance); the National Nosocomial Infections Surveillance System (NNIS)/National Healthcare Safety Network (NHSN) established by the Centers for Disease Control and Prevention in the US) are used as one major source of information. The second source of information constitute national or regional studies meeting the above mentioned criteria. The scope and design as well as the strengths and weaknesses of surveillance studies have been critically reviewed previously [10–12].

2. Mode of Action and Mechanisms of Resistance

2.1. Interaction with Bacterial Type II Topoisomerases. Fluoroquinolones are the only class of antimicrobial agents in clinical use that are direct inhibitors of bacterial DNA synthesis. Fluoroquinolones inhibit two bacterial enzymes, DNA gyrase and topoisomerase IV, which have essential and distinct roles in DNA replication. The quinolones bind to the complex of each of these enzymes with DNA; the resulting topoisomerase-quinolone-DNA ternary complex subsequently leads to the generation of double-stranded breaks in DNA and blocks progress of the DNA replication enzyme complex. Ultimately, this action results in damage to bacterial DNA and bacterial cell death [13–16].

Resistance to quinolones occurs by mutation in chromosomal genes that encode the subunits of DNA-gyrase and topoisomerase IV (altered target mechanism), and that regulate the expression of cytoplasmic membrane efflux pumps or proteins that constitute outer membrane diffusion channels (altered permeation mechanism). Several excellent and comprehensive reviews have been published summarizing the current knowledge about the mode of action and resistance mechanisms of fluoroquinolones; the reader is kindly referred to these publications for further reading (e.g., [16–21]). Furthermore, reduced target expression has been described as another mechanism leading to low level quinolone resistance [22].

2.2. SOS Response and Autoinduction of Fluoroquinolone Resistance. Repair mechanisms are activated as a consequence of inhibition of bacterial type II topoisomerases. Any DNA-damage triggers the production of various repair proteins by activating an SOS gene network [23–27]. The SOS system is composed of more than 40 genes and is controlled by regulatory proteins RecA and LexA. RecA provides a signal for induction of SOS response, while LexA functions as a repressor; binding the gene repressor LexA unmasks its autoproteolytic activity, so that the 40 SOS genes are no longer repressed. The LexA binding site is located in the sequence upstream from qnrB (but not qnrA or qnrS), so that qnrB is regulated by the SOS-system, too, in response to DNA damage [28]. In addition, it has been shown recently that the SOS response promotes qnrB expression [29]. The peptide QnrB protects bacterial DNA-topoisomerases from quinolone inhibition and provides low-level quinolone resistance (see below Section 2.3. “plasmid mediated fluoroquinolone resistance”). The Qnr-determinants facilitate the emergence of high-level resistance. In E. coli, this latter effect depends on the increased mutation ability conferred by the nonessential polymerases Pol II, Pol IV, and Pol V on LexA-cleavage-mediated derepression of their respective genes (polB, dinB, and umuDCC; 106). Thus, qnrB-mediated quinolone resistance and increased mutation ability are two events triggered by the same signal, namely, the SOS response. Quinolone resistance gene qnrB is upregulated by ciprofloxacin in a RecA/LexA-dependent manner, so that quinolone resistance development is an integral part of their mode of action in qnrB harboring bacteria. Ciprofloxacin resistant mutants could be elicited much more frequently in LexA positive wild-type strains than in LexA mutant strains [30, 31]. Vice versa, preventing LexA cleavage renders bacteria unable to evolve resistance to fluoroquinolones [30, 31]. Furthermore, SOS response induces persistence to
fluoroquinolones [32]. These results support the notion that fluoroquinolones are not only mere selectors of resistant variants but that bacteria themselves play an active role in the mutation of their own genomes. Quinolone resistance is not only acquired via target site mutations, but also via the SOS system by derepression of genes whose products increase mutation rates. In general, interference with bacterial stress response may reduce the emergence of resistance [33]. Furthermore, it was shown recently that ciprofloxacin stimulated SOS independent recombination of divergent DNA sequences in E. coli. Thus, fluoroquinolones increase genetic variation via a second, SOS independent mechanism [34]. This mechanism, too, may favour acquisition, evolution, and spread of resistance determinants.

Not only DNA damaging agents like quinolones trigger the SOS response. Beta-lactams interfering with penicillin binding protein 3 [35, 36], zidovudine or trimethoprim [37], and rifampin [30] activate the SOS gene network as well. These data demonstrate that induction of SOS response by any of these drug classes facilitates persistence and evolution of resistance in general. Thus, it may be speculated that these agents, too, may affect quinolone activity and/or resistance development via the SOS promoted expression of qnrB. Furthermore, the SOS system contributes to the spread of antibiotic resistance by promoting horizontal dissemination of antibiotic-resistance genes [38] or mutations.

2.3. Plasmid Mediated Fluoroquinolone Resistance. The genetic information for target site or efflux resistance mechanisms is commonly chromosomally encoded. However, the emergence of plasmid-mediated and thus transferable fluoroquinolone resistance has also been reported; several mechanisms are known: 1. Qnr, 2. Aminoglycoside acetyltransferase AAC(6′)-Ib-cr, 3. OqxAB, QepA [39–44].

The emergence of plasmid-mediated quinolone resistance was first found in strains of Klebsiella pneumoniae in one region of the United States in 1998 [45] and shown to be due to a member of the pentapeptide repeat (PPR) family of proteins Qnr (later named QnrA). In the following years, several distantly related plasmid mediated Qnr determinants were described in Enterobacteriaceae (QnrB, QnrC, Qnr D, QnrS) [46, 47]. They have been identified worldwide and are almost always associated with the production of expanded spectrum β-lactamases [48–50]. Qnr-like peptides (sharing an amino-acid identity with QnrA of 16 to 22%) have been found in the Gram-positive bacteria Mycobacterium tuberculosis, M. smegmatis, and M. avium [51], E. faecalis [52], and in E. faecium, Listeria monocytogenes, C. perfringens, C. difficile [53]. Recently, a new chromosomally encoded quinolone resistance gene of the PPR family has been identified in Stenotrophomonas maltophilia and has thus been named Smqnr [54]; a smaqnr gene has been found in Serratia marcescens [55].

Qnr interacts with DNA-gyrase and topoisomerase IV to prevent quinolone inhibition [39, 56]. Qnr protein causes nalidixic acid resistance and reduced susceptibility to or low-level fluoroquinolone resistance [56]. Qnr-genes have been found in ciprofloxacin-susceptible isolates as well as quinolone resistant isolates, suggesting that their presence promotes higher level resistance due to chromosomal mutation, as has been shown in the laboratory. Therefore, the presence of qnr genes in clinically relevant species of both, Gram-positive and Gram-negative bacteria may foster quinolone resistance development. Furthermore, qnrA and qnrB genes are usually integrated into integrons which harbor other antibiotic resistance genes such as β-lactamases or aminoglycoside inactivating enzymes. Although qnrS-genes are not harbored by integrons, they are associated with transposons containing TEM-1 type β-lactamases [57]. Consequently, the association of genes encoding for quinolone resistance and resistance to other drug classes like β-lactams and aminoglycosides favour the selection and dissemination of fluoroquinolone resistant strains by chemically unrelated drug classes, and vice versa, of β-lactam or aminoglycoside-resistant strains by fluoroquinolones (the close correlation between extended spectrum β-lactamases (ESBL) production and quinolone resistance is discussed in the chapters on fluoroquinolone resistance).

Qnr genes were also found on the chromosome of an environmental water bacterium, Shewanella algae. Other qnr homologs have been found in the genome sequences of several Vibrio spp. and Photobacterium profundum suggesting that water-borne Vibrionaceae may have been the source of and may constitute a reservoir for the qnr genes [58–60]. Recently it was demonstrated in vitro that the plasmid borne Shewanella algae qnr gene could be transferred to Enterobacteriaceae [58].

Another plasmid-encoded quinolone resistance determinant was identified, a variant of the aac(6′)-Ib gene encoding an aminoglycoside acetyltransferase. The bifunctional aminoglycoside and fluoroquinolone active variant AAC(6′)-Ib-cr catalyzes acetylation of both drug classes [61]. The variant enzyme has acquired the ability to acetylate ciprofloxacin and norfloxacin and reduces ciprofloxacin’s activity fourfold [62, 63]. Moxifloxacin and levofloxacin are not acetylated due to the absence of a piperezinyl substituent at position C-7. Interestingly, the first ciprofloxacin resistant clinical isolate (S. marcescens) was isolated from a patient treated in the pre-quinolone era with a β-lactam and an aminoglycoside; the pre- and post therapy MICs of ciprofloxacin were 0.06 and 4 mg/L, respectively. This strain produced an aminoglycoside acetyltransferase and showed changes in the outer-membrane composition [64]. AAC(6′)-Ib-cr may be more widespread than Qnr-determinants. Both, Qnr- and AAC(6′)-Ib-cr-production are associated with the ESBL production, thus, representing a second mechanism of co-selection of drug-resistance due to exposure to chemically unrelated agents.

Most recently, a third type of plasmid-mediated quinolone resistance has been identified: the quinolone efflux pumps OqxAB and QepA [42–44, 65, 66]. The OqxAB- and QepA-proteins confer resistance to hydrophilic fluoroquinolones like norfloxacin, ciprofloxacin, and enrofloxacin, causing a 32- to 64-fold increase in MICs [65–68]. QepA extrudes in addition to quinolones a narrow range of agents such as erythromycin, ethidium bromide, and acriflavine; OqxAB exports a wider range of agents like ethidium bromide, tetracyclines, chloramphenicol, trimethoprim,
olaquindox, and the disinfectants like triclosan [57, 68, 69].

The problem is that the qepA gene and an aminoglycoside ribosome methyltransferase are part of a transposable element [66], so that there is a potential of selection of QepA determinants by aminoglycosides and vice versa aminoglycoside resistance by quinolones; the same holds true for aac(6′)Ib gene mediated resistances. Extrusion of chemically unrelated agents by efflux-pumps represents a third mechanism of cross-resistance. In conclusion, fluoroquinolone resistance can emerge even in the absence of exposure to this drug class as several coselection mechanisms favour the emergence of quinolone resistance.

Additional, unknown mechanisms of quinolone resistance must exist as known chromosomally-and plasmid-mediated resistance mechanisms plus the presence of the multidrug efflux pump AcrAB were detected in just 50–70% of high-level quinolone resistant E. coli clinical isolates with MICs up to 1,500-fold higher than expected [70].

2.4. Additional Resistance Mechanisms. Any antibacterial agent interacting with an intracellular target must traverse the bacterial cell-wall and cytoplasmic membrane to reach the target. Once taken up, most antibacterials are actively effluxed. Therefore, fluoroquinolones, too, are affected by permeation barriers and efflux pumps, either in association with target modifications or on their own.

As mentioned above, many Gram-positive and Gram-negative fluoroquinolone-resistant mutant strains do not show any mutation in the quinolones resistance determining region (QRDR). For example, 70% of E. coli mutants recovered from besifloxacin selection plates were characterized by the absence of classical QRDR mutations [71] and 61% high-level ciprofloxacin-resistant isolates of E. coli accumulated lower levels of ciprofloxacin than the wild type, in addition to the gyrA mutations found in all of them [72]. Furthermore, chemically unrelated substances like cyclohexane, salicylate, and tetracycline affected fluoroquinolone susceptibilities of E. coli, too; 21 of 57 high level fluoroquinolone-resistant clinical isolates of E. coli showed tolerance to cyclohexane, suggesting an elevated broad spectrum efflux activity [73]. Multiple antibiotic resistance (mar) genes cause an efflux of a variety of chemically unrelated compounds including different drug classes of antibacterials and antiseptics.

Additional nontopoisomerase resistance mechanisms that are not under mar control can change quinolone resistance patterns. The nfxB gene codes for an altered outer cell membrane protein F, thereby decreasing quinolone entry into the cell [79]. In addition, soxRS gene products, which are involved in bacterial adaptation to superoxide stress, affect fluoroquinolone activity, too [73].

Various combinations of target enzyme alteration, diminished antibiotic accumulation, and efflux are often seen in fluoroquinolone-resistant E. coli, other Enterobacteriaceae and nonfermenters [72, 80]. Cross-resistance between fluoroquinolones and antibacterials of chemically unrelated drug classes is associated with the increased expression of efflux pumps because of their limited substrate specificity. For example, MexAB confers resistance to nonfluorinated and fluoroquinolones, tetracycline, and chloramphenicol, Mex CD confers resistance to fluoroquinolones, erythromycin, trimethoprim, and triclosan, Mex EF confers resistance to the latter plus chloramphenicol, imipenem, and triclosan, and Mex XY confers resistance to fluoroquinolones, erythromycin, and aminoglycosides. Several comprehensive reviews have summarized the impact of fluoroquinolone-efflux and resistance [80–83]. Consequently, a fluoroquinolone resistant or even multidrug-resistant phenotype can easily be selected by an exposure to a broad range of chemically unrelated drug classes, thus, representing the fourth type of cross-resistance.

These examples illustrate the complexity of fluoroquinolone resistance mechanisms, selection by fluoroquinolones and coselection of resistance by chemically unrelated classes of antibacterials and antiseptics.

3. Fluoroquinolone Resistance Epidemiology

3.1. Urinary Tract Infections. The first quinolone used clinically, that is, nalidixic acid, was classified as an “urinary antiseptic”; previous nonfluorinated quinolones were almost exclusively used for treatment of lower urinary tract infections (UTIs). The fluorinated quinolones are characterized by more marked antibacterial activity against uropathogens, so that ciprofloxacin resistant E. coli strains isolated from female outpatients were almost nonexistent (<1%) till the mid-1990s; resistance to ciprofloxacin increased slowly from 1.2% in 1998 to 2.5% in 2001 [84]. The same holds true for uropathogenic E. coli isolated from male and in-patients, respectively, with a trend towards higher resistance rates among elderly patients [85, 86]. However, the NAUTICA (North American Urinary Tract Infection Collaborative Alliance) study revealed that ciprofloxacin resistance increased to 5.5% in 2004 [87]. Likewise, uropathogens studied during the years 1996 and 2009 in the province of British Columbia demonstrated an increase in fluoroquinolone resistance. The resistance rates in E. coli and K. pneumoniae increased from <2% in 1996 to ≥20% in 2009; the resistance rates of fluoroquinolones for P. mirabilis remained almost constant throughout the years at ≤2%. Enterococci demonstrated frequently resistance against fluoroquinolones although resistance rates decreased between 2002 and 2009 [88].
3.1.1. Community Acquired Urinary Tract Infections. Data summarized in Table 1 demonstrate that fluoroquinolone resistance ranges from 2.2% to 69% for strains isolated from patients with uncomplicated, community acquired UTI (CAUTI) and even up to 98% for strains from patients with complicated CAUTIs. Likewise, ESBL production ranged from 2.6% to 100%. Both, fluoroquinolone resistance and ESBL production were highest in the Asia-Pacific region and moderate to low in Europe and North America. The clonality of the isolates has rarely been examined, although high numbers of ESBL producers may indicate that a few clones may predominate amongst the isolates studied (see below, Section 3.1.3). Furthermore, data summarized in Table 1 indicate that on the one hand the relative numbers of ESBL producers per centre is high whereas on the other hand the total numbers of isolates is still quite small. For example, 100% of the ESBL positive strains were fluoroquinolone-resistant; but this corresponds to 11.8% of the total number of isolates studied [89]. The high relative figures of fluoroquinolone resistant ESBL-producers—which are often mentioned in the abstract instead of the total numbers—may mask the prevalence of fluoroquinolone resistance in uropathogens.

The risk for acquisition of CAUTIs caused by ESBL-positive E. coli and the distribution of the ESBL enzyme types was determined in a prospective cohort study [90]. A total of 510 patients with CAUTIs caused by Gram-negative bacteria were included in the study. ESBL producers were detected in 6.3% of uropathogenic E. coli isolated from uncomplicated UTIs and 17.4% of E. coli isolates from complicated UTIs (P < 0.001), most of which (90.2%) were found to harbour CTX-M-15. According to multivariate analysis, more than three urinary tract infection episodes in the preceding year (OR 3.8, P < 0.001), use of a β-lactam antibiotic in the preceding 3 months (OR 4.6, P < 0.001) and prostatic disease (OR 9.6, P < 0.004) were found to be associated with ESBL positivity. The percentages of isolates with simultaneous resistance to trimethoprim-sulphamethoxazole, ciprofloxacin, and gentamicin were found to be 4.6% in the ESBL negative group and 39.2% in the ESBL positive group (P < 0.001) [90].

Comprehensive reviews of the worldwide emergence of ESBL producing Enterobacteriaceae indicate that 1st their numbers increase continuously, 2nd ESBL production is diverse, scatters geographically, and originates from both, community associated-as well as healthcare associated infections [91–93], 3rd most of the community isolates are multi-resistant, [92, 94], 4th many isolates are often genetically related and clonal spread has been reported frequently [95–101], 5th the pandemic multiresistant, community associated clone ST 131 is highly prevalent and contributes to 30% to 60% to all fluoroquinolone resistant E. coli [93, 102].

Clearly, the continuously increasing prevalence of ESBL-producing Enterobacteriaceae isolated from out-patients is alarming. However, several studies indicate that the prevalence of ESBL-producing, fluoroquinolone-resistant CAUTI-pathogens may be low. The ECO. SENS (E. coli sensitivity) project is a Pan-European survey of the antimicrobial susceptibilities of pathogens from uncomplicated UTIs. Data published in 2003 demonstrate that overall ciprofloxacin resistance in the 2,478 E. coli strains collected amounted to 2.3%, ranging from 0% in Austria and Sweden to 5.8% and 14.7% in Portugal and Spain, respectively. Ciprofloxacin-resistance rates in P. mirabilis, Klebsiella spp. and other Enterobacteriaceae were 2.1%, 1.0%, and 0.8%, respectively [103]. The ARESC (Antimicrobial Resistance Epidemiological Survey on Cystitis) study revealed that in uropathogens collected in nine European countries and Brazil from 2003 to 2006 ciprofloxacin resistance in E. coli was recorded in >10% of all the isolates in Brazil, Spain, Italy, and Russia; in the remaining European countries, ciprofloxacin resistance ranged from 1.4% in France to 6.7% in Poland [104–106]. As national parts of the ARESC study, 335 and 650 uropathogens, respectively, were isolated most recently from German and Spanish patients with uncomplicated cystitis; fluoroquinolone resistance amounted to 7.7% and 11.9%, respectively [107, 108], thus, indicating that fluoroquinolone resistance did not increase as compared to the previous study period. ESBL production was neither specified in the ECO. SENS nor the ARESC study.

3.1.2. Healthcare Associated Urinary Tract Infections. Fluoroquinolone resistance ranged from 6.3% to 62% in Gram-negative strains and 20% and 100% of the methicillin-susceptible S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA), respectively, as well as 59% of the Enterococci isolated from patients with complicated, healthcare associated UTI (HAUTI) (Table 1). In general, uropathogens from patients admitted to tertiary care hospitals are less fluoroquinolone susceptible than those from out-patients. Clearly, patients admitted to tertiary care hospitals suffer from chronic diseases, urologic surgery, recurrent infectious diseases necessitating antibacterial therapy prior to the actual study, and so forth, so that one or several risk factors favor development of resistance. High rates of fluoroquinolone resistance were found in patients with HAUTIs evaluated in the emergency department [109, 110] and in nursing home residents [111]. Horizontal transmission of one, or few predominating clone(s) in nursing home residents is frequent [2].

3.1.3. Association between Fluoroquinolone Resistance and Production of Extended Spectrum β-Lactamases. Although production of extended spectrum β-lactamases (ESBLs) was not analysed in these studies, it may well be that the increase in both, fluoroquinolone resistance and ESBL-production, are closely associated [112]. ESBLs gained prominence and started to spread among uropathogens in North America at the time when these surveillance studies have been performed.

Since the early 1990s, E. coli isolates that produce CTX-M type ESBLs have emerged as a serious cause of UTIs in the community [113–116]. E. coli strains that produce CTX-M ESBLs, primarily found in community sources, are becoming widely prevalent worldwide [95–97, 113]. For example, in Spain a threefold rise in community-onset UTIs caused by ESBL-producing E. coli over a 3-year period from 0.47% (17 of 3,617 isolates) in 2000 to 1.7% (44 of 2,600
Table 1: Worldwide prevalence of fluoroquinolone-resistant uropathogens. Data in columns four and five represent total numbers of isolates studied; data in columns six to nine represent fluoroquinolone-resistant isolates in percent of total; figures in column ten show ESBL-positive isolates in percent of total isolates studied; figures in columns eleven and twelve represent ESBL-positive or negative fluoroquinolone-resistant isolates in percent of total numbers of ESBL-positive isolates.

<table>
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<th>Species</th>
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<th>n cUTI</th>
<th>HAUTI uUTI</th>
<th>HAUTI cUTI</th>
<th>CAUTI uUTI</th>
<th>CAUTI cUTI</th>
<th>ESBL pos % of total</th>
<th>% FQ res. of ESBL pos</th>
<th>% FQ res. of ESBL neg</th>
<th>Ref.</th>
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<td>—</td>
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<td>—</td>
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<td>HAUTI uUTI</td>
<td>HAUTI cUTI</td>
<td>CAUTI uUTI</td>
<td>CAUTI cUTI</td>
<td>ESBL pos % of total</td>
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<td>% FQ res. of ESBL neg</td>
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<td>6.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.25</td>
<td>98.75</td>
<td>—</td>
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</tr>
<tr>
<td>E. coli</td>
<td>USA</td>
<td>08/08 to 03/09</td>
<td>253</td>
<td>—</td>
<td>38</td>
<td>—</td>
<td>—</td>
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<td>[506]</td>
</tr>
<tr>
<td>E. coli</td>
<td>USA</td>
<td>08/08 to 03/09</td>
<td>102</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>E. coli</td>
<td>USA</td>
<td>08/08 to 03/09</td>
<td>357</td>
<td>—</td>
<td>10.0</td>
<td>3.8</td>
<td>2.0</td>
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<td>—</td>
<td>[109]</td>
</tr>
<tr>
<td>E. coli</td>
<td>NLD</td>
<td>1997</td>
<td>332</td>
<td>—</td>
<td>29.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>11.2</td>
<td>88.8</td>
<td>—</td>
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<td>1997</td>
<td>332</td>
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<td>29.0</td>
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<tr>
<td>E. coli</td>
<td>NLD</td>
<td>2004 + 2009</td>
<td>565</td>
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<td>3.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>[508]</td>
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<tr>
<td>E. coli</td>
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<td>01/04 to 12/09</td>
<td>420</td>
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<td>12.0</td>
<td>—</td>
<td>—</td>
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<td>96.5</td>
<td>—</td>
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<td>345</td>
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<td>K. pneumoniae</td>
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<td>09/05 to 06/06</td>
<td>45</td>
<td>—</td>
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<td>37.8</td>
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<td>[263]</td>
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<tr>
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<td>09/05 to 06/06</td>
<td>51</td>
<td>—</td>
<td>0.0</td>
<td>—</td>
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<td>—</td>
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</tr>
<tr>
<td>MSSA</td>
<td>CAN</td>
<td>09/05 to 06/06</td>
<td>16</td>
<td>—</td>
<td>6.3</td>
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<td>95.1</td>
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<td>[264]</td>
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<tr>
<td>K. pneumoniae</td>
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<td>01/08 to 12/08</td>
<td>98</td>
<td>—</td>
<td>12.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.2</td>
<td>96.8</td>
<td>—</td>
<td>[264]</td>
</tr>
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</table>

uUTI: uncomplicated urinary tract infection; cUTI: complicated urinary tract infection; HA-UTI: Healthcare associated urinary tract infection; CA-UTI: community acquired urinary tract infection; ESBL: extended spectrum β-lactamase; Ref: reference; *: total number of isolates studied in both sampling periods; ESP: Spain; GRC: Greece; PRT: Portugal; TUR: Turkey; LBN: Lebanon; IND: India; PAK: Pakistan; PRK: South Korea; HKG: Hong Kong; ZAF: South Africa; RUS: Russian Federation; USA: United States of America; NLD: The Netherlands; CHE: Switzerland; CAN: Canada.
isolates) in 2003 was reported, 31% of which (or 0.54% of the total isolates) were resistant to ciprofloxacin [117]. A nationwide study performed in Spain in 2000 revealed that 93% of the ESBL-producing K. pneumoniae strains were isolated from inpatients, whereas 51% of ESBL-producing E. coli strains were isolated from outpatients [118]. Risk factors for the acquisition of ESBL-producing E. coli in non hospitalised patients with uncomplicated urinary tract infections (uUTIs) were diabetes mellitus (odds ratio (OR) = 5.5), previous fluoroquinolone use (OR = 7.6), previous hospital admission (OR = 18.2), and older age in male patients (OR = 1.03) [119]. A prospective cohort study in 510 patients with CAUTIs caused by Gram-negative bacteria revealed that ESBL producers were detected in 6.3% of uropathogenic E. coli isolated from uncomplicated UTIs and 17.4% of E. coli isolates from complicated UTIs (P < 0.001), most of which (90.2%) were found to harbour CTX-M-15 [19]. According to multivariate analysis, more than three urinary tract infection episodes in the preceding year (OR 3.8, P < 0.001), use of a β-lactam antibiotic in the preceding 3 months (OR 4.6, P < 0.001) and prostatic disease (OR 9.6, P < 0.004) were found to be associated with ESBL positivity. The percentages of isolates with simultaneous resistance to trimethoprim-sulphamethoxazole, ciprofloxacin, and gentamicin were found to be 4.6% in the ESBL-negative group and 39.2% in the ESBL-positive group (P < 0.001) [90]. As the CTX-M type is most common among the CAUTI pathogens it is conceivable that many of these isolates may be genetically related. More than two thirds of unduplicated E. coli strains isolated from patients admitted to nine different Portuguese hospitals in three different regions were ESBL producers; all of the CAUTI pathogens produced the CTX-M-15 type β-lactamase. Three quarters of the ESBL producers belonged to one genetic cluster, indicating countrywide dissemination of one single clone [120]. An analysis of selected E. coli strains isolated in eight European countries during 2003 to 2006 from patients with uncomplicated cystitis displaying reduced ciprofloxacin susceptibility revealed that 55 different biochemical profiles could be distinguished; although this finding indicates a substantial heterogeneity, about one third of all isolates belonged to two clonal groups O25:H4-ST 131 and O15:K52:H1. ESBL production was detected in 8.1% of all isolates, CTX-M-15 being the most common; strains belonging to the two predominant clonal groups had ciprofloxacin MICs of 16 and ≥32 mg/L, respectively [91, 102, 121]. Point source dissemination of ESBL-producers is frequent in patients with uUTIs. E. coli ST 131 was the most predominant group and accounted for 23.1% and 46%, respectively, of ESBL-positive isolates overall [91, 102]. Nearly all ST 131 isolates were ciprofloxacin resistant. The intercontinental pandemic spread of the ciprofloxacin-resistant E. coli O25:H4:ST 131 clonal group producing CTX-M-15 has been described worldwide in hospital and community settings [122, 123]. The sudden worldwide increase of ESBL-producing E. coli is mostly due to the single CTX-M-15 positive clone ST131; foreign travel to high-risk areas, such as the Indian subcontinent, play in part a role in the spread of this clone across different continents [124]. The isolation of a multidrug-resistant E. coli strain of sequence type ST 131 from an 8-month old girl with severe septic arthritis and contagious osteomyelitis and her healthy mother demonstrates that within household transmission contributes to the dissemination of the ST 131 clonal group, too [125]. Furthermore, plasmid-mediated fluoroquinolone resistance determinants including CTX-M-15 were common in areas of high fluoroquinolone consumption [126] and in nursing home residents in whom a single multiresistant clone spread [127].

3.1.4. Risk Factors for and Impact of Prescribing Habits on Emergence of Fluoroquinolone Resistance. The impact of prescribing of ciprofloxacin on the emergence of fluoroquinolones resistance in uropathogenic E. coli was analysed in 72 general practices in the west of Ireland. Over a 4.5 year period (from April 2004 to September 2008) susceptibility and prescribing data were collected and analyzed by a multivariable model with ciprofloxacin-resistance as outcome and prescribing as predictor. The analysis revealed that in “mean” practices with one prescription per month ciprofloxacin resistance was low (3%) whereas in practices with 10 prescriptions per month ciprofloxacin resistance amounted to 5.5% [128]. Analogous effects were noted in patients with CAUTI monitored over a 6-year period in Denver, Colo, USA [129]. In 1999, the initial therapy of uUTI was switched to levofloxacin. The prescriptions increased from 3.1 to 12.7 per 1,000 visits; in parallel, fluoroquinolone resistance increased from 1% to 9%. Risk factors for the acquisition of fluoroquinolone resistant E. coli were hospitalization (or for each week of hospitalization = 2.0), and levofloxacin use within the previous year (OR 5.6). Similar risk factors were identified by others, too [130–134]. Additional factors favoring the selection of resistant uropathogens are poor adherence to treatment guidelines [135] and dispensing of antibacterials without prescription [136].

Another aspect is worth mentioning and relevant for prescribing policies, hygiene strategies, and resistance statistics. A study on the evolution of quinolone resistance in Barcelona, Spain from 1992 to 1997 revealed that the prevalence of fluoroquinolone resistance in the feces of healthy people was unexpectedly high, 24% in adults and 16% in children, although not used in the pediatric population [137, 138]. The carriage rate was higher than the fluoroquinolone resistance rates among patients with healthcare and community acquired infections (8.3% and 9% in 1992 versus 18% and 17% in 1996, resp.). Increasing fluoroquinolone resistance rates in commensal E. coli in children were found in North as well as South America, Africa, and Asia, too [139–145]. Among pediatric blood-stream isolates there was an association between fluoroquinolone resistance and ESBL production [141]. Similarly, the Chinese isolates from pediatric patients are characterized by a high prevalence of plasmid-mediated quinolones resistance; 4.1% were positive for qnr and 8.2% for aac(6’)-Ib-cr genes known to confer low level fluoroquinolone resistance or to inactivate ciprofloxacin, but not moxifloxacin [145]. Isolates from children had relatively high prevalences of ciprofloxacin resistance in the 1990s already although the
use of ciprofloxacin in pediatric populations was approved for treatment of inhalational anthrax (post exposure) in August 2000 and for treatment of cUTI in March 2004. The fluoroquinolone resistance in children could be due to the transmission of resistant isolates between adults and children in families, daycare, or school settings and in previous years to the use of fluoroquinolones in poultry populations. These findings demonstrate that spread of fluoroquinolone resistance due to environmental contamination as well as person to person transmission contributes to an increase in the numbers of resistant isolates independent from selection of resistant strains in diseased patients; this phenomenon may bias resistance statistics. Analogues findings will be reported below for RTI-pathogens. Furthermore, these findings indicate that treatment of fluoroquinolone-naïve patients, that is, those who should not have been treated in previous years because of their age, may nevertheless carry primed bacteria which may develop high-level fluoroquinolone resistance quite rapidly during treatment.

Conclusion. These data demonstrate that most of the uropathogens causing uncomplicated UTIs in outpatients are still susceptible to fluoroquinolones, but considerable regional differences in drug susceptibility patterns exist with alarming rates of fluoroquinolone-resistant and/or ESBL-producing uropathogens in the Asia-Pacific region and India. Because of the very close correlation between ESBL-production and fluoroquinolone resistance in uropathogenic Enterobacteriaceae, fluoroquinolone susceptibility is still high in all those geographic regions in which ESBL producing Gram-negative community-acquired uropathogens are infrequent. Pathogens causing HAUTIs or cUTIs in nursing home patients are less susceptible to fluoroquinolones. Because of the considerable variability of susceptibility patterns in different countries, local epidemiological data are critical in the empiric management of UTIs, in particular in patients with risk factors and nursing home residents. Furthermore, fluoroquinolones exert a MRSA selective potential and exhibit negative epidemiological effects resulting in the selection of multiresistant pathogens. Therefore, fluoroquinolones should be used with caution even in patients with CAUTI and in particular in patients with HAUTI [146–148].

3.2. Respiratory Tract Infections

3.2.1. Community Acquired Respiratory Tract Infections. Although a number of significant pathogens like Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila are associated with community acquired respiratory tract infections (CARTIs) in all age groups [149–151], S. pneumoniae is the most frequent one. In the past, three major RTI surveillance studies, the Alexander Project [152], the RTI component of SENTRY [153], and PROTEKT (prospective resistant organism tracking and epidemiology for the ketolide telithromycin, sponsored by Aventis Pharmaceuticals) [154] have provided invaluable data on global antimicrobial resistance in CARTI-pathogens. Penicillin resistance rates in pneumococci varied from 71% in South Korea, 57% in Hong Kong, and 40% to 50% in France, Spain, and Japan, whereas no penicillin-resistance was detected in Indonesia or the Netherlands [155–161]. Likewise, macrolide resistance among RTI pathogens varied from 0% to 41% [155, 157]. In Taiwan, penicillin and/or macrolide and/or trimethoprim/sulfamethoxazole-resistance amounts to 72%, 92%, and 76%, respectively [162]. Interestingly, even in these “hot spots” of penicillin- and/or macrolide and/or trimethoprim/sulfamethoxazole resistance like Asia or Spain where fluoroquinolone use is high and low doses are administered frequently, rates of fluoroquinolone resistance remain low.

It is important to note that in the studies quoted below the definitions of ciprofloxacin and levofloxacin resistance are based on two different resistant breakpoints, that is, ≥4 mg/L for ciprofloxacin and ≥8 mg/L for levofloxacin.

No levofloxacin-resistant pneumococci were detected in eight Asian countries from 2002 to 2004 [163, 164]. In Taiwan, only 0.6% of pneumococcal isolates collected from 2000-01 were resistant to levofloxacin [154]; by 2003, 3% of isolates in Taiwan were resistant to levofloxacin [162]. From 192 pneumococcal isolates collected in China from 2001 to 2002, 6.8% were resistant to levofloxacin; 4.2% were resistant to moxifloxacin [160, 165]. In 2008, 6.5% of S. pneumoniae isolated from hospitalized patients in Bangkok, Thailand, were resistant to ofloxacin [166]. A national surveillance study in Japan from 1994 to 2002 revealed that levofloxacin resistance rates were below 2% and were stable throughout the observation period; however, an increase in levofloxacin resistance rates from 0% in 1998 to 9.5% in 2000, and 4.8% in 2002 was found among penicillin-resistant pneumococci [161]. Recently, four highly levofloxacin-resistant pneumococci (MIC > 32 mg/L) were detected in Japan among 345 strains collected in Gifu prefecture from May 2006 to July 2006 [167]. Also in Spain, fluoroquinolone resistance rates remain low, ranging from 0.6 to 7% for ciprofloxacin [168–172]. A recent nationwide susceptibility study collected in 34 laboratories 2,559 S. pneumoniae isolates from patients with community acquired pneumonia (CAP); only 2.2% and 0.5% of these isolates were ciprofloxacin and levofloxacin resistant [173].

Fluoroquinolone resistance is rare in North America. Surveillance studies in the United States from 1987 to 2009 demonstrated low rates of resistance (0.1 to 1.3%) to levofloxacin [174–195] and to moxifloxacin (0.1%; 216). From 27,828 isolates of S. pneumoniae collected in the US during 4 consecutive respiratory seasons from 1998 to 2002, only 1.3% were levofloxacin-resistant [181] although ciprofloxacin has been used in the US since 1987 and has thus exerted a selective pressure on S. pneumoniae. Likewise, the prevalence of fluoroquinolone resistance in Canada remained low from 1998 to 2009. Although total per capita outpatient use of fluoroquinolones increased during this 10-year period, levofloxacin and moxifloxacin resistance remained unchanged at <2% in the >26,000 isolates collected [196]. However, a trend for rising levofloxacin resistance from <0.5% to >3% was noted in some regions of North America [85, 179, 180, 190, 191]. The GLOBAL (global landscape on the bacterial activity of levofloxacin) surveillance programme is an initiative intended to detect susceptibility
changes in CARTI pathogens in Europe and Asia [196]. Results from the programme revealed that the susceptibility profiles of 2,395 \textit{S. pneumoniae} isolated from 1997 when the study was initiated till 2007 remained unchanged, that is, $\geq 96\%$ in Asia and $\geq 98.6\%$ in Europe [196]. Analogous data were obtained in the course of the Alexander Project, collecting isolates from Europe, Middle East, Asia, South and North America [157, 197]. Likewise, the MOXIAKTIV study (a German multicenter study with 29 participating laboratories) demonstrated that 99.3\% of the pneumococci were moxifloxacin and levofloxacin susceptible and the MICs of moxifloxacin were as low as those of the prelaunch isolates [198]. These in vitro findings are mirrored by the low prevalence of fluoroquinolone-resistant strains isolated from patients with pneumococcal pneumonia. In 1.2\% of the isolates a first step mutation was detected and 6.7\% exhibited an efflux phenotype, despite high fluoroquinolone usage [199].

Increasing fluoroquinolone resistance in pneumococci paralleled increased usage of fluoroquinolones in general or 2nd generation quinolones in particular [178, 199–201]. Occasionally, fluoroquinolone resistance resulted in clinical failures in patients with pneumococcal pneumonia having been previously treated empirically with oral fluoroquinolones [160, 185, 202–204]. In total, there were 20 ciprofloxacin and levofloxacin treatment failures reported till January 2005 and reviewed by Fuller and Low [204]. A pretherapy isolate was available in five cases only with MICs ranging from 1 mg/L to 16 mg/L; MICs for the during-therapy isolates ranged from 4 mg/L to $>32$ mg/L [204]. Thus, the question cannot be answered if resistance may have developed during therapy resulting in clinical failure. This question was recently addressed by Orr et al. [205] who investigated in a tertiary referral hospital in England in 865 patients the incidence and epidemiology of levofloxacin-resistant pneumococci. In six patients a shift towards reduced levofloxacin-susceptibility or -resistance was recorded. Five patients had acquired a new distinct strain and one patient only harboured the same clone [205]. This study revealed that levofloxacin pneumococcal resistance still is uncommon and that in vivo fluoroquinolone resistance development is very rare. If it does occur, strain replacement accounts for the majority of cases. A limitation of this study is that all isolates of \textit{S. pneumoniae} from any body site were eligible for inclusion in the study, irrespective of whether the patient has been treated with a fluoroquinolone or not. Furthermore, hospital guidelines recommend to treat severe community acquired pneumonia with levofloxacin plus intravenous benzylpenicillin [205]. High-level levofloxacin-resistance (MIC $> 8$ mg/L) developed under levofloxacin-treatment in eight out of 164 patients with chronic obstructive pulmonary disease whose pretherapy isolates were susceptible [206]. A fatal outcome was described in another patient with chronic obstructive pulmonary disease who was infected with a \textit{S. pneumoniae} strain with a preexisting \textit{parC} mutation; the MIC of levofloxacin for this strain was 1 mg/L, so that the mutation passed unnoticed and the strain was classified as susceptible [207]. A \textit{P. aeruginosa} infection was treated successfully with oral ciprofloxacin in another COPD patient in whom a ciprofloxacin resistant but moxifloxacin-susceptible (MIC 0.125 mg/L) \textit{S. pneumoniae} strain was isolated subsequently; this strain harbored a \textit{parC} mutation [208].

The prevalence of first-step fluoroquinolone-resistant \textit{S. pneumoniae} mutants is increasing [195, 200, 208]. Although the subtle changes in MICs of 3rd generation fluoroquinolones for primed bacteria remained within the susceptible range in most CARTI-isolates, many isolates contained a single \textit{gyrA} or \textit{parC} mutation, which prime the bacteria to acquire additional mutations within the quinolone resistance determining region (QRDR) conferring high-grade fluoroquinolone resistance [209–211]. Three up to 30\% of clinical pneumococcal isolates contain mutations in the \textit{gyrA} and/or \textit{parC} loci [179, 209, 212, 213].

These data demonstrate that many pneumococcal isolates with first-step fluoroquinolone resistance may pass unnoticed in routine susceptibility testing because of the high resistance breakpoints. This theory has been proven by two in vitro screening tests [214, 215]. Previously, the resistant breakpoints for ciprofloxacin and levofloxacin were $>4$ mg/L and $>8$ mg/L, respectively. Actually, the resistant breakpoints of ciprofloxacin and levofloxacin for \textit{S. pneumoniae} defined by EUCAST are $\geq 2$ mg/L. The EUCAST provides two comments in this context: 1st, wild type \textit{S. pneumoniae} are not considered susceptible to ciprofloxacin, and 2nd the breakpoints for levofloxacin relate to high dose therapy. However, high levofloxacin doses, that is, 750 mg once or 500 mg twice daily, are rarely administered, so that an extrapolation from the categorization “susceptible” due to in vitro breakpoint based susceptibility testing to an advise on therapy in the patient is limited. Two case reports describing levofloxacin treatment failures confirm the limited predictability of routine in vitro susceptibility testing. First, a 71-year-old male patient was hospitalized due to pneumococcal pneumonia. The pretherapy isolate was levofloxacin susceptible with a MIC of 2 mg/L although it had a point mutation in \textit{gyrA}; the post-therapy isolate had an additional mutation in \textit{gyrA}. The patient was treated with 500 mg iv for 13 days; on day 4 intravenous clarithromycin was added and on day 14 treatment was changed. Initial treatment with levofloxacin failed due to an acquisition of a second mutation in \textit{parC} resulting in a MIC of 16 mg/L [216]. Second, a 79-year-old male patient was hospitalized with bacteremic pneumonia caused by levofloxacin susceptible \textit{S. pneumoniae} with a MIC of 1 mg/L. The patient was treated with 500 mg levofloxacin iv. After initial improvement fever reappeared on day 4, so that amoxicillin was added; but the clinical condition failed to improve and the patient died one day later. This pathogen had a preexisting mutation in \textit{parC}; the post-therapy isolate had an additional mutation in \textit{gyrA} [207]. Both patients had apart from the advanced age additional risk factors like COPD and others.

These clinical examples confirm that first step mutants of \textit{S. pneumoniae} are 1st phenotypically considered to be susceptible and 2nd are primed to acquire additional QRDR mutations conferring high-grade fluoroquinolone resistance resulting in clinical failure [217]. As most first step mutants pass routine susceptibility testing unnoticed they are not effectively detected in surveillance studies, so that these may
be biased. Consequently, routine susceptibility testing of suspicious cases at least should be modified, for example, by using a second fluoroquinolone like ciprofloxacin as an indicator for the acquisition of a first mutation. Furthermore, it should be considered to use a more potent antipseudomococal fluoroquinolone than levofloxacin, for example, a “respiratory fluoroquinolone” like a C-8-methoxyquinolone. Moxifloxacin and gatifloxacin, or gemifloxacin may have replaced the previous fluoroquinolones in the treatment of CARTIs. 2nd, treatment guidelines may have been adapted recommending the use of a second agent like benzylpenicillin in, for example, elderly or patients with other risk factors. 3rd, information about patient history and previous antibiotic use is crucial for determining appropriate empirical therapy [190, 223]. 4th, acquisition of some parC and gyrA mutations may impose a fitness cost to the first step fluoroquinolone-resistant strains, although equivocal data have been generated [224–226].

*Haemophilus influenzae* is generally highly susceptible to fluoroquinolones; global surveillance studies demonstrated that susceptibility to fluoroquinolones remained at or near 100% [197, 227–230]. Resistant isolates have been recovered occasionally [230–237]. For example, during the 1997 through 1998 SENTRY-programme four (0.13%) fluoroquinolone-resistant *H. influenzae* strains were identified [238]. The strains were genetically distinct and had different gyrA mutations. Furthermore, clonal outbreaks of fluoroquinolone-resistant *H. influenzae* were observed in long-term care facilities [239–241] and in elderly in Japan [242].

Because of the occurrence of fluoroquinolone-resistant strains, Hirakata et al. [243] screened a total of 400 *H. influenzae* strains isolated in 138 hospitals throughout Japan. The strains were consistently very susceptible to ciprofloxacin with MICs ranging from ≤0.03 to 0.25 mg/L; the majority of strains was inhibited by ciprofloxacin concentrations ≤0.03 mg/L. Therefore, the authors examined the strains (*n* = 37 out of 400) with MICs 0.06 mg/L and higher for QRDR mutations. From these, one ciprofloxacin-resistant isolate (MIC = 16 mg/L) and 31 ciprofloxacin-susceptible isolates (MICs, 0.06 to 0.5 mg/L) had amino acid changes in their QRDRs. Moreover, 9.8% of the 363 highly ciprofloxacin-susceptible isolates (MIC ≤ 0.03 mg/L) had mutations in their QRDRs, particularly in the case of β-lactamase positive amoxicillin-clavulanate resistant isolates [243].

These data clearly demonstrate that—indeed to *S. pneumoniae*—many fluoroquinolone-susceptible *H. influenzae* have acquired QRDR mutations; these strains pass routine susceptibility testing unnoticed, but are primed to mutate further. Routine susceptibility testing of suspicious cases at least should be modified, for example, by using nalidixic acid as an indicator for the acquisition of a first mutation [228, 244]. The presence of *H. influenzae* with reduced levofloxacin-susceptibilities in kindergarten children in Hong Kong is alarming; the MICs of nalidixic acid and levofloxacin were 64–128 mg/L and 0.125 mg/L, respectively [245]. Likewise, the report about a levofloxacin treatment failure in a patient with *H. influenzae* pneumonia is worrying. The 71-year-old patient has been treated with 500 mg levofloxacin once daily; after 7 days the clinical condition had not improved and therapy was changed. Levofloxacin MICs for *H. influenzae* isolated from blood-cultures and bronchial aspirates at day 7 amounted uniformly to 16 mg/L and all the isolates had changes in the QRDR [246].

*M. catarrhalis* remains fluoroquinolone susceptible at almost 100%, although resistant strains have been detected in a very few single cases [197, 228, 229, 231, 247]. Two treatment failures with clonally unrelated resistant strains have been reported in patients at risk [248].
Conclusion. The three major pathogens causing CARTI are fluoroquinolone-susceptible to almost 100%. However, first-step mutants have been detected frequently not only in treated patients but also in healthy individuals and even children. Such isolates are primed to mutate to high-level fluoroquinolone resistance during subsequent fluoroquinolone-treatment.

3.2.2. Nosocomial Respiratory Tract Infections. In treatment guidelines and reviews, nosocomial pneumonia is further differentiated into healthcare associated pneumonia (HCAP), hospital acquired pneumonia (HAP), and ventilator associated pneumonia (VAP) [249–254]. Bacterial pathogens most frequently associated with HCAP, HAP, and VAP are methicillin-susceptible and -resistant S. aureus (MSSA, MRSA), Pseudomonas aeruginosa, H. influenzae, K. pneumoniae, E. coli, and occasionally S. pneumoniae and Acinetobacter spp. [255]. Resistance surveillance studies differentiating the origin of isolates tested according to pneumonia categories are almost nonexistent; resistance-rates are quoted in very general terms even in some of the guidelines quoted above. Therefore, information compiled below summarises susceptibility data for invasive pneumococci or pathogens isolated from sputa obtained preferably from ICU-patients. S. pneumoniae isolates were from patients with invasive as well as noninvasive diseases in eight European countries and Latin America were examined in the PneuWorld Study from 2001 to 2003. Susceptibility testing revealed that fluoroquinolone resistance rates ranged from 0% in Austria, Switzerland, and Belgium to 0.9% in Germany and 1.2–1.3% in Italy and Portugal [256]. From the bacteremia pneumococci isolated from 1999 to 2007 in the UK and Ireland, 14.3% were resistant to ciprofloxacin [257]. Rates of levofloxacin-resistance in invasive S. pneumoniae collected by the Centers for Disease Control and Prevention (CDC) Active Bacterial Core Surveillance Program Network (ABCS) remained stable throughout the years at about 0.3% to 0.43% [258, 259]. This finding contradicts reports of seven-valent pneumococcal conjugate vaccine-driven expansion of fluoroquinolone resistant clones [164, 260, 261]; others have hypothesized that a decrease in fluoroquinolone resistance among invasive pneumococci may be due to reduction of absolute numbers of isolates within the vaccine serotypes [262]. Nevertheless, the potential for the clonal expansion and dissemination of fluoroquinolone-resistant strains obtained from the ABCS program has been demonstrated [175]. Clonal spread of levofloxacin resistance in invasive S. pneumoniae isolates was identified in Madrid, Spain [176]. Likewise, clonal spread of levofloxacin-resistant pneumococci could be demonstrated in strains from Hong Kong, whereas strains collected in Okinawa, Japan, were not clonally related [177].

All S. pneumoniae blood-isolates sampled in 2005-2006 and 2008 from Canadian emergency room- and ICU patients were ciprofloxacin susceptible [263, 264]. Ciprofloxacin-resistance among MSSA- and MRSA-blood isolates collected in 2008 amounted to 8% and 81.6%; ciprofloxacin-resistance in respiratory isolates was 11%, and 95.6%, respectively [264]. All H. influenzae blood-isolates were ciprofloxacin-susceptible [263]. Ciprofloxacin-resistance rates in E. coli, P. aeruginosa, and K. pneumoniae isolated from blood were 21.6%, 16%, and 4.3%, respectively. Eight percent of these E. coli isolates were ESBL producers. Ciprofloxacin resistance in respiratory isolates of E. coli, P. aeruginosa, and K. pneumoniae was 31.7%, 18.4%, and 4.5%, respectively [264]. Pathogens isolated from ICU patients not categorized in patients with/without nosocomial RTIs showed variable fluoroquinolone resistance [265]. Pathogens were collected in the USA (283 sites), Canada (87 sites), France (63 sites), Germany (169 sites), and Italy (48 sites) from January 2000 till December 2002. Pneumococci were highly susceptible in all geographic regions. In MSSA and MRSA, fluoroquinolone resistance varied from 4.8% in Canada to 8% in Germany, and from 90.6% in France to 9.6% in Germany, respectively. In E. coli, fluoroquinolone resistance ranged from 6.5% in France to 12.7% in Italy; resistance in K. pneumoniae ranged from 7.2% in Canada to 9.9% in Italy; resistance in P. aeruginosa ranged from 22.9% in Germany to 76.7% in Italy [265]. In ten Asian countries, ciprofloxacin resistances in P. aeruginosa, E. coli, and K. pneumoniae isolated from HAP- and VAP-patients ranged from 4–44%, 26–80%, and 13–68% [266]. Similar rates were reported for Gram-negative species isolated from Indian VAP-patients [267].

Fluoroquinolones have in the past shown good activity against A. baumannii [268]; however, over the past decade there has been a constant rise in fluoroquinolone- and multidrug resistance [269, 270]. Fluoroquinolone resistance in Acinetobacter spp. isolated from HAP- and VAP-patients in ten Asian countries varied from 23.2 to 92% [250]. Fluoroquinolone resistance in Acinetobacter spp. isolates from North American and European ICU-patients with/without nosocomial RTIs ranged from 25.9% in Canada to 76.7% in Italy [265]. Fluoroquinolone resistance in A. baumannii isolates sampled from sputa and tracheal aspirates of ICU patients in a tertiary care hospital in Ankara amounted to 86% [271].

Conclusion. Pneumococci and haemophilia isolated from HCAP, HAP, and VAP patients are almost all fluoroquinolone-susceptible. MSSA and in particular MRSA are frequently fluoroquinolone-resistant. Enterobacteriaceae and nonfermenters are variably fluoroquinolone-resistant, so that the regional resistance pattern has to be considered prior to the use of a fluoroquinolone in the treatment of nosocomial pneumonias.

3.2.3. Cystic Fibrosis. One of the most striking aspects of natural history of P. aeruginosa and its association with cystic fibrosis (CF) is the adaptation and heterogeneity exhibited by the organisms as colonisation of the lung develops to a chronic state. In the early stages of colonisation the P. aeruginosa population is usually homogeneous with respect to colonial morphology, antigenicity and drug susceptibility. Later, however, considerable heterogeneity is observed and the P. aeruginosa population shows a considerable degree of heterogeneous antimicrobial susceptibility with MICs ranging over a broad range from hyper susceptibility to
high-level resistance [272–275]. *P. aeruginosa* being heteroresistant to all relevant antibacterials including ciprofloxacin have been described by these authors. For example, the MIC of ciprofloxacin for one genetically homogeneous isolate as determined by routine methods was 0.5 mg/L prior to ciprofloxacin therapy; however, population analysis revealed that hypersusceptible subpopulations were present at high frequencies and subpopulation with MICs up to 16 times the MIC for the entire population were present at frequencies ranging from $2 \times 10^2$ to $5 \times 10^{-2}$. The population analysis of the post-exposure isolate showed that the hypersusceptible subpopulations have been eradicated; the subpopulations with 2 to 8 times the pre therapy MIC occurred at frequencies of approx $1 \times 10^{-2}$ and the subpopulations with 32 and 64 times the pre therapy MIC were present with frequencies of $4 \times$ and $2 \times 10^{-4}$ [275]. Consequently, there is a high probability in CF patients that multiple subpopulations of *P. aeruginosa* with a broad range of MICs will exist, so that in principle a single MIC value for the entire population does not exist. Therefore, selection of colonies for susceptibility testing [276] as well as routine susceptibility testing of mixed morphotypes of *P. aeruginosa* yields inaccurate results; for example, predictability of ciprofloxacin susceptibility and resistance of a single isolate from a CF patient was 87.0% and 41.7%, respectively [277]. Thus, the value of conventional susceptibility-testing of bacteria isolated from CF patients is questionable [278]. In addition, fluoroquinolone resistance emerges in the first few days of therapy and viable counts of the pathogen are reduced minimally. Therefore, the fluoroquinolone used to treat CF patients must exert pleiotropic effects on *P. aeruginosa*; ciprofloxacin, for example, inhibits quorum sensing [279] or modulates immune response [280, 281]. However, it was demonstrated recently in vitro and in patients that antivirulence interventions based on quorum-sensing inhibition with a macrolide diminish natural selection towards reduced virulence and therefore may increase the prevalence of more virulent genotypes [282]. Thus, it has to be studied clinically in CF patients if a fluoroquinolone may exert quorum sensing inhibition at all, and if the virulence of the pathogen may be affected or not.

Furthermore, a common feature of *P. aeruginosa* isolated from CF patients is the very high prevalence of mutator (or hypermutable) strains in contrast to those with an up to 1,000-fold lower spontaneous mutation rate of strains isolated from patients with acute infections [283, 284]. Such hypermutator strains persisted and even amplified (50,000-fold) in contrast to nonhypermutator strains despite adequate, that is, administration of standard doses, exposure to ciprofloxacin [285]. Recent studies have shown that mutators may affect modulation of virulence factors, genetic adaptation to the growth environment in the infected patient, persistence and perhaps also transmissibility [286].

Conventional susceptibility testing—thus not considering the heterogeneous susceptibility pattern of the subpopulations—of *P. aeruginosa* isolates from CF patients revealed that ciprofloxacin resistance in Europe ranged from 13.7% in Bulgaria [287] to approximately 30% in the UK, Spain, Germany, and Italy [288–291]; 37.4% of the US isolates were ciprofloxacin-resistant [292]. Mucoid strains tended to be less ciprofloxacin susceptible than non mucoid isolates [290]; 27.8% of the non mucoid and 35.3% of the mucoid isolates were susceptible to ciprofloxacin.

Patients with cystic fibrosis suffer from *S. aureus* infections, too. MRSA carriage and infection are becoming increasingly common among CF patients. It appears that healthcare associated-MRSA predominate, but asymptomatic community associated-MRSA colonisation may be a predictor of disease [293]. The emergence and spread of a specific MRSA isolate in Marseille, France, is warning. This well-adapted multiresistant isolate is closely related to the vancomycin resistant strain Mu50 and spreads rapidly in CF patients [294]. This strain is also characterized by the presence of an antibiotic inducible (e.g., imipenem, tobramycin, ciprofloxacin) bacteriophage which may result in high frequency transfer and the unintended promotion of spread of virulence and resistance determinants.

The presence of hypermutable *P. aeruginosa* and MRSA in CF patients is a threat to the patient and a challenge for any antibacterial agent.

*Conclusion.* *P. aeruginosa* colonising and infecting CF patients are geno- and phenotypically highly heterogeneous, so that any routine susceptibility testing and resistance surveillance studies are misleading. It is an inevitable consequence of therapy that preexisting resistant subpopulations will be selected, so that resistance will develop rapidly under treatment.

### 3.3. Skin and Skin Structure Infections

Acute bacterial skin and skin structure infections (ABSSSI) are typically monomicrobial and caused by *S. aureus* and *S. pyogenes* which are also the most common pathogens in complicated bacterial skin and skin structure infections (cSSSI) which are frequently polymicrobial. However, Gram-negative and anaerobic microbes become more prevalent. The most common Gram-negative organisms in cSSSIs include *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *E. cloacae*. The most common anaerobes isolated are typically Prevotella, Bacteroides, and Peptostreptococcus species [295, 296].

Although *S. pyogenes* were and are still highly susceptible to fluoroquinolones, low incidences (≤8%) of ciprofloxacin resistance have been found globally; fluoroquinolone resistance in Japan is almost nonexistent [297–315]. In Belgium, fluoroquinolone resistance increased from 2.8% to 13.1% from 2003 to 2005 and decreased thereafter to 8.9% in 2006 [307]. It is important to note, that in Belgium approx. 55% of the fluoroquinolone-resistant isolates were recovered from children aged less than 16 years [307]. Although fluoroquinolones are contraindicated in children, ciprofloxacin is often used off-label for select life-threatening conditions. Furthermore, older and thus cheap fluoroquinolones are used topically for treatment of otitis media with otorrhoea through tympanostomy tubes in paediatric patients.

In the early days of fluoroquinolone development and clinical use the fluoroquinolones were regarded as potential alternatives to MRSA therapy with a β-lactam or vancomycin. This was due to the fact that resistance to
fluoroquinolones has rarely emerged in the various staphylococcal infection models. Especially in experimental endocarditis caused either by MSSA or MRSA fluoroquinolones proved effective and were not associated with the development of fluoroquinolone resistance in most of the models. In addition, their in vivo activity was equivalent or even superior to that of vancomycin or imipenem [2, 316, 317].

Unfortunately, staphylococci acquire resistance to antibacterials quite rapidly as they are genetically highly variable [318]. The determinant for methicillin resistance is located on the so-called staphylococcus cassette chromosome mec (SCCmec). Some of the SCCmec elements contain additional genes for antibiotic resistance encoding for aminoglycoside-, tetracycline-, and macrolide-lincosamide-streptogramin-resistance [319, 320]. Furthermore, HA-MRSA tended to develop fluoroquinolone resistance more frequently than MSSA [321, 322]. This phenomenon may be due to the fact that on the chromosomal map of the S. aureus genome the mecA gene is located between protein A and DNA gyrase genes. Therefore, mutations in the gyrase may have an effect on the expression of mecA in HA-MRSA strains [323] and some cell wall associated proteins such as protein A and fibronectin binding proteins [324, 325]. Thus, almost any antibacterial drug class has a methicillin-resistance selective potential [326–328], so that strains of HA-MRSA are almost always multidrug-resistant.

Therefore, fluoroquinolone resistance developed rapidly in the early days of fluoroquinolone therapy in HA-MRSA. Hospital admissions in the US for ABSSSI caused by fluoroquinolone resistant MRSA increased from 29% between 2000 and 2004 [329] to 70.3% in 2008 [330]. In addition, fluoroquinolone-resistant HA-MRSA were spread horizontally as were HA-MRSA as such, so that nowadays neither the 2nd-nor the 3rd-generation fluoroquinolones represent alternatives for treatment of HA-MRSA infections [5, 331–338].

In recent years, the emergence of CA-MRSA has complicated the treatment of even ABSSSI [296, 332, 333]. CA-MRSA strains differ in several ways from HA-MRSA strains like composition of the SCC mec, the carriage of plasmids encoding resistance to antibacterials of other drug classes and in their associated pathogenicity factors [336]. In contrast to multidrug resistance usually seen in HA-MRSA strains, antibiotic resistance in CA-MRSA is most often limited to macrolides [319, 337–340], so that it has previously been proposed that some 3rd-generation fluoroquinolones could be useful in the treatment of CA-MRSA, since the causative pathogens were usually susceptible to even ciprofloxacin [341–346]. But recently mupirocin, tetracycline, clindamycin, and moxifloxacin (and thus to any commercially available fluoroquinolone) resistance development has been reported [347, 348]. The clone USA 300 became the predominant strain type in the USA and has spread to Europe, South America, and Australia [347, 349, 350]. The lineage USA 100 is frequent, too [351]. Fluoroquinolone resistance in isolates recovered from a phase IV study in patients with cSSSI in the USA and EU from 2004 to 2007 was high; 100% of USA 100-isolates and 42.6% of USA 300 isolates were resistant to gatifloxacin [351]. Community MRSA isolates in general, and the USA 300 clone in particular are increasingly multidrug resistant, with resistance profiles recently broadening to include clindamycin, tetracycline, mupirocin, and fluoroquinolone agents, in addition to the β-lactams; occasionally, community isolates also display reduced susceptibility to vancomycin or resistance to gentamicin or trimethoprim-sulfamethoxazole [352].

Pathogens collected from 27 USA and 28 EU medical centers in 2009 causing cBSSSI were variably susceptible to fluoroquinolones: levofloxacin resistance in the USA/EU amounted to 70.3%/84.1% in MRSA, 11.1%/5.4% in MSSA, 54.2%/52.3% in coagulase-negative staphylococci, 0.9%/0.0% in β-hemolytic streptococci, 13.6%/1.1% in viridans streptococci, 37%/29.2% in E. faecalis, 24.7%/21.8% in E. coli, 11%/13.3% in Klebsiella spp., and 20.8%/8.0% in P. mirabilis [353]. These resistance rates are within the same range as those reported in the late 1990s and 2001–2004 for Gram-negative and Gram-positive aerobic pathogens isolated in North America, Latin America, and Europe from skin and soft tissues [354–356], thus, indicating that resistance rates did not change substantially over time.

Of 175 anaerobic bacteria isolated in the late 1990s from bacterial skin and soft-tissue infections, 27% were levofloxacin-resistant [357]. All Peptostreptococcus species isolated from hospitalised patients with diabetic foot wound infection were susceptible to levofloxacin and moxifloxacin; resistance (5–7%) was found in isolates of B. fragilis, Bacteroides ovatus, and Prevotella species collected in 1999 to 2002. [358, 359]. Against B. fragilis, moxifloxacin’s MIC90 was 1.0 μg/mL. Against other Bacteroides species, the MIC90 was 2–4 μg/mL. Moxifloxacin was least active against Fusobacterium species other than F. nucleatum (MIC90, 8 mg/L). Among anaerobic species isolated from patients with moderate to severe diabetic foot infections from 2001 to 2004 in the USA, 24% were fluoroquinolone resistant [356]. In detail, moxifloxacin resistance rates were: 43% B. fragilis group, 10% Fusobacterium spp., 2% Porphyromonas spp., Gram-positive cocci 18%, and Gram-positive rods 12% [283]. As levofloxacin is less active against anaerobes, resistance rates were correspondingly higher. Of all infection sites, decubitus ulcer isolates had the highest resistance rates [360].

Conclusion. In principle, a 3rd generation fluoroquinolone is well suited for treatment of polymicrobial SSSIs because of its broad antibacterial spectrum. Fluoroquinolone resistance rates among pathogens causing skin and soft tissue infections is low in MSSA, and streptococci, moderate in Gram-negative aerobes as well as Gram-positive anaerobes, and high in CA-MRSA, HA-MRSA, and Gram-negative anaerobes. This heterogeneous susceptibility pattern may limit the use of fluoroquinolones in the treatment of ABSSSIs and cBSSSIs.

3.4. Intra-Abdominal Infections. The Surgical Infection Society and the Infectious Diseases Society of America (IDSA) have recently published guidelines for the diagnosis and treatment of complicated intra-abdominal infections (IAIs). E. coli, Enterococcus spp., Bacteroides fragilis, and other Bac- teroides species are the most common pathogens associated
with intra-abdominal infections [7, 361]. Intra-abdominal infections are commonly due to mixed aerobic and anaerobic populations, so that a clinically effective regimen has to cover both, the aerobic Enterobacteriaceae and Enterococci, as well as the anaerobic bacteria.

Several surveillance studies have demonstrated that there is a global trend toward decreasing susceptibilities of anaerobes to antibacterial agents since two decades. Although the rates of resistance show clinically important variations between continents, countries, and counties, almost all drug classes—except metronidazole—like beta-lactams including the carbapenems, clindamycin and quinolones lose activity against anaerobes. A dramatic loss of antianaerobic activity of the carbapenems, clindamycin and quinolones lose activity between continents, countries, and counties, almost all drug classes—except metronidazole—like beta-lactams including the carbapenems, clindamycin and quinolones lose activity against anaerobes. A dramatic loss of antianaerobic activity of the carbapenems, clindamycin and quinolones lose activity between continents, countries, and counties, almost all drug classes—except metronidazole—like beta-lactams including the carbapenems, clindamycin and quinolones lose activity against anaerobes. A dramatic loss of antianaerobic activity of the carbapenems, clindamycin and quinolones lose activity between continents, countries, and counties, almost all drug classes—except metronidazole—like beta-lactams including the carbapenems, clindamycin and quinolones lose activity against anaerobes. A dramatic loss of antianaerobic activity of the carbapenems, clindamycin and quinolones lose activity between continents, countries, and counties, almost all drug classes—except metronidazole—like beta-lactams including the carbapenems, clindamycin and quinolones lose activity against anaerobes. A dramatic loss of antianaerobic activity of the carbapenems, clindamycin and quinolones lose activity between continents, countries, and counties, almost all drug classes—except metronidazole—like beta-lactams including the carbapenems, clindamycin and quinolones lose activity against anaerobes. A dramatic loss of antianaerobic activity of

The continuously increasing quinolone resistance amongst anaerobes is surprising because of a variety of reasons: First, previous fluoroquinolones like norfloxacin, ofloxacin, ciprofloxacin, levofloxacin were not used clinically for treatment of anaerobic infections. Nevertheless, surveillance testing in the US between 1994 and 1996, that is, prior to the launch of the first antianaerobic quinolone trovafloxacin, revealed that quinolone resistance ranged from 3% to 8%. Second, quinolone resistance rates increased in 1997 to 13%, although trovafloxacin was approved in December 1997. Quinolone resistance continued to increase to 15% in 1998. Despite the limited use of trovafloxacin in 1998 and its relegation to a restricted therapeutic category in June 1999, frequencies of quinolone resistance increased further, peaking at 25% in 2001 [360, 368]. Furthermore, it has been speculated that third, older fluoroquinolones like norfloxacin, ciprofloxacin, ofloxacin, and levofloxacin may have fostered quinolone resistance development [360, 368]. However, this hypothesis is not convincing either as the older fluoroquinolones have been heavily used since their launch. Furthermore, the older fluoroquinolones are almost inactive against anaerobes [369, 370]. Although very high total concentrations are achieved in the faeces, free and thus antibacterial active concentrations, are low as quinolones are highly and tightly bound to cell debris, DNA, cellulose, and other fecal matter [371]; therefore, norfloxacin, ciprofloxacin, ofloxacin, and levofloxacin suppress growth of fecal aerobic Gram-negative rods but do not affect significantly the anaerobic flora. Anaerobes with increased MICs or quinolone resistance have rarely been isolated from patients during or shortly after a quinolone treatment [372]. Thus, the driving forces for quinolone resistance development are at present unknown.

Very heterogeneous data on quinolone resistance amongst anaerobes have been reported, ranging for example from 27% to 50% in the US [363, 373], and 44.4% in Canada [374], 15% to 25% in Spain [275, 364, 365, 375], and 0% to 32% in Greece [364, 365, 376]. This heterogeneity of susceptibility data within and between countries—which is typical for aerobic species, too—may reflect marked differences in 1st, the patient populations from whom the isolates were obtained (health-care versus community acquired infections—which, however, is almost always not specified); 2nd, prior antibiotic exposure; 3rd, the patient populations admitted to either tertiary care—or primary care centres; 4th, the sites of isolation; 5th, the limited number of participating centres. Overall, 9% of the European B. fragilis group isolates were moxifloxacin-resistant in 2002; a moderate increase to 13.6% was noted in 2009. Geographical differences were detected in 2009, too, with higher resistance rates for moxifloxacin in Scandinavian (21.4%) and Eastern (11.3%) than in Mediterranean countries (5.4%) [365].

A most recent German study with 32 participating centers revealed that moxifloxacin MICs for anaerobes are by one to two titration steps higher than prior to its launch. Resistance rates ranged from 10% to 22% for various anaerobic species except B. vulgatus, with 59% of the isolates being resistant. It became evident, too, that resistance rates are higher in isolates obtained from 1st, tertiary care versus primary care centres, 2nd, patients admitted to the ICU versus standard care, and 3rd, health care versus community acquired infections [377].

The resistance epidemiology of quinolone resistance among anaerobes has to be complemented with resistance figures in Enterobacteriaceae isolated from patients with intra-abdominal infections in order to cover the entire spectrum of potential pathogens. In general, the situation in Asia is alarming as resistance-rates surpass 60% of the isolates being resistant to ampicillin-sulbactam or a quinolone and producing ESBL [378–384]. ESBL production in E. coli, K. pneumoniae, or K. oxytoca was highly variable in the Asia-Pacific region ranging in total from 4.4% in New Zealand to 77.4% in India. Only 17% and 27% of the ESBL-producing E. coli and K. pneumoniae strains, respectively, were susceptible to ciprofloxacin [379]. In Europe, 11.8% and 17.9% of the E. coli and K. pneumoniae strains isolated from patients with intra-abdominal infections were ESBL producers [385], ranging from 0% in Lithuania and Switzerland to 30% in Greece. From these, 70% or 78% and 50% or 70% of the community or hospital acquired E. coli and K. pneumoniae strains were ciprofloxacin resistant. In the US, ESBL production was detected in 4.7% and 17.5% of E. coli and K. pneumoniae isolates, respectively.

From these, 33% and 19% were susceptible to ciprofloxacin [386]. Ciprofloxacin resistance in a worldwide collection of IAI pathogens amounted to 22.8% in E. coli, and 15.6% in K. pneumoniae [387]. Both, ESBL production and fluoroquinolone resistance remained high or even increased in 2009-2010 in the Asia-Pacific region, Europe, North- and Latin America; ESBL producers were more frequently isolated from elderly [388–393]. These data confirm—in analogy to the UTI-isolates—the very close correlation between ESBL production and fluoroquinolone resistance in Enterobacteriaceae causing IAI. Consequently, fluoroquinolone susceptibility is still high in all those geographic regions in which ESBL-producing Gram-negative bacilli are infrequent. Another clinically relevant finding—again in agreement with UTIs—is that fluoroquinolone resistance was much lower in strains isolated from patients with community acquired intra-abdominal infections than in those from hospital-acquired infections.
Conclusion. Fluoroquinolone resistance is high amongst aerobic and anaerobic intra-abdominal pathogens. Therefore, the Infectious Diseases Society of America and the Surgical Infection Society published a guideline in late 2009 recommending that antibacterials to be used in the empiric treatment of even community-acquired intra-abdominal infections including mild to moderate infections should be active against both, aerobic and anaerobic pathogens. Consequently, the use of quinolones should be restricted unless resistance rates are lower than 10% [7, 361, 394].

3.5. Sexually Transmitted Diseases. Infections caused by Neisseria gonorrhoeae and Chlamydia trachomatis are the most frequent ones among reportable bacterial sexually transmitted diseases (STD) gonorrhoea, syphilis, and chancroid. Infections due to Chlamydia spp. were diagnosed almost 4-times more frequently than infections due to Neisseria spp. (409.2 cases versus 110.7 cases in the USA in 2009). Chlamydia spp. diagnosis increased by 2.8% in 2009 as compared to 2008, and by nearly 20% since 2006, likely due to expanded screening. Gonorrhea cases declined by 11% overall. Syphilis cases increased, too, while chancroid cases have declined steadily till 2001 and are fluctuating since then. However, Haemophilus ducreyi, the causative organisms of chancroid, is difficult to culture, so that this condition may be substantially underdiagnosed. In general, there were large disparities by age, race, and geographical distribution [395–397]. Pelvic inflammatory disease (PID) is a common and serious complication of some sexually transmitted diseases. Two-thirds of cases are considered to be due to sexually-transmitted infections caused by N. gonorrhoeae and C. trachomatis; one-third (particularly in older women) are commonly polymicrobial. Other pathogens such as Mycoplasma genitalium and bacterial vaginoses pathogens (e.g., Gardnerella vaginalis, Mycoplasma hominis, Mobiluncus spp. and other anaerobes) may cause PID, too. Actinomycetes are part of the normal vaginal flora and a rare cause of PID. Therefore, management of PID must take into account in particular the three major pathogens N. gonorrhoeae, C. trachomatis, and M. genitalium.

Coinfections with C. trachomatis and N. gonorrhoeae are common among young heterosexual patients with gonorrhoea. Therefore, all treatments for STD/PID should cover both, N. gonorrhoeae and C. trachomatis as well as anaerobes [398, 399], and M. genitalium has to be considered [397].

3.5.1. Neisseria gonorrhoeae. Initially, Neisseria spp. was extremely susceptible to fluoroquinolones with ciprofloxacin MICs of \( \leq 0.008 \) mg/L. However, low level resistance (0.06–0.5 mg/L) was reported shortly after its launch [400–402], followed soon by high-level resistance (MICs of ciprofloxacin >1.0 mg/L) associated with treatment failures [402–404]. High-level fluoroquinolone resistance is first, more likely to emerge in areas with a high prevalence of low-level resistance; second, it is spread intercontinentally by travellers and an intercity spread and transmission has been reported; third, mono-as well as multi-clonal spread of quinolone-resistant isolates has been reported [405–407].

Typically, several different strain types can be identified by using molecular typing methods; for example, 24 different quinolone-resistant strain types were identified among the isolates having caused an outbreak in California, but only four of these were considered outbreak types and comprised 66% of all the isolates [408]. Furthermore, importation (often repeated importation) of one or a few clone(s) and ultimate introduction into established sexual networks have caused the emergence and spread of resistant gonococci rather than de novo emergence as a result of selection by quinolone use or misuse [409].

Both, low-level and high-level fluoroquinolone resistance has been reported from all parts of the world (reviewed in [410]). Ciprofloxacin resistance in N. gonorrhoeae is highest in Asia; resistance rates in China vary from 40 to 100%, depending on the region studied [410–413]. In Korea, ciprofloxacin resistance increased from 9% in 1992, to 84% in 1999, and to 90.5% in 2004 and 83% in 2006 [414, 415]. In India, ciprofloxacin resistance varied from 80.7% in 2002, 97.2% in 2004 to 88.6% in 2006 [416–418]. In Pakistan, ofloxacin–resistance increased from 0% in 1998 to 92.5% in 2009 [419] and ciprofloxacin resistance in isolates collected from 2007 to 2010 in Iran amounted to 53.2% [420]. In Kenya, ciprofloxacin resistance increased from 9.5% in 2007 to 50% in 2009 [421] and ranged in other African countries from 0% in Malawi or Mozambique to 41.9% in South Africa [422]. Quinolone resistance in the Western Pacific Region ranged in 2009 from ≤1.5% Fiji, Papua New Guinea and New Caledonia via 35% to 42% in New Zealand and Australia up to >95% in Vietnam, Philippines, and Hong Kong, [423]. Gonococcal resistance to ciprofloxacin in the Netherlands, Italy, Greece and in Norway exceed 40% [424–427] which is in the same range as the data previously reported by the “European Surveillance of Sexually Transmitted Infections” (ESSTI) [428] and by the EUROSURVEILLANCE [429]. However, ciprofloxacin resistance increased to 63% in 17 European countries participating in the European gonococcal antimicrobial surveillance programme, 2009 [430] and was high in the eastern part of the WHO European region, too [431]. Rates of ciprofloxacin resistance amongst the gonococcal isolates rose in Canada from 1.4% in 2001 to 28% in 2006/2007 [432, 433] and the US from <1% in 2001 to 6.7% in the first half of 2006 to 14.8% in 2007, decreasing to 13.5% and 9.6% in 2008 and 2009, respectively, increasing again to 12.5% in 2010 [395, 434, 435]. Consequently, quinolones are not recommended as first-line therapy of N. gonorrhoeae infections anymore [435–438]. The emergence of multi-drug resistant N. gonorrhoeae reduces the treatment-options further [439–444] as such isolates are resistant to quinolones, third generation cephalosporins, and additional agents.

3.5.2. Chlamydia trachomatis. Quinolone resistance in C. pneumoniae has not been described clinically or even in vitro; however, high-level resistance to ofloxacin, sparfloxacin, and ciprofloxacin occurred in C. trachomatis upon serial exposure to subinhibitory quinolone-concentrations [445–449]. However, spontaneous mutation frequencies resulting in moxifloxacin resistance were very low or even nonexistent;
Exposure of C. trachomatis serovars L2 and D resulted in emergence of quinolone resistance at a frequency of 2.0–2.2 x 10^{-8} in serovar L2 only, whereas no resistant clones could be elicited in serovar D [450]. It is important to note that these experiments were performed under routine conditions, that is, a relatively high inoculum (approx. 2.7 x 10^9 inclusion forming units) was exposed to the drug, whereas the bacterial load at the focus of infection is much lower thus reducing the likelihood of drug-induced resistance selection. Nevertheless, fluoroquinolone-resistant strains of C. trachomatis have been isolated occasionally [449, 450]. Fluoroquinolone resistance elicited in vitro in C. trachomatis serovar L2 was due to a single nucleotide point mutation in gyrA, while no mutations were found in gyrB, parC, or parE genes; no QRDR mutations could be detected in the fluoroquinolone-resistant clinical isolates [451].

3.5.3. Mycoplasma genitalium. Surveillance studies for antimicrobial resistance in general and fluoroquinolone resistance in particular are not existent as culturing of this species from clinical specimens is extremely difficult. Acquired resistance to fluoroquinolones has been described in single cases. Analysis of the gyrA and parC genes of M. genitalium isolated from 6 men in whom levofloxacin therapy failed [452] revealed that in one patient a ParC amino acid change could be detected in the pre- as well as post-therapy isolate, whereas in another patient a ParC-mutation was detectable in the post-therapy isolate only. No QRDR mutations could be detected in strains isolated from the remaining four patients [453]. M. genitalium clinical isolates from 28 men with nongonococcal urethritis positive for M. genitalium were analyzed by PCR. QRDR-mutations were found in five of these 28 isolates; no alterations were detected in the remaining isolates [454]. The two studies quoted above were performed by noncultural methods, so that no MICs could be determined; thus, an association between QRDR mutations and fluoroquinolone resistance and persistence cannot be proven. Furthermore, it should be considered that the patients in whom persisters could be isolated had been treated with low levofloxacin doses (100 mg t.i.d. for 14 days); in addition, levofloxacin is characterized by a moderate activity against M. genitalium while for example, C8-methoxyquinolones are ten times as active [455, 456].

Conclusion. Resistance of N. gonorrhoeae to antimicrobials continues to increase worldwide, although considerable geographical variations in resistance exist. Therefore, fluoroquinolones are not recommended as first-line therapy of N. gonorrhoeae infections anymore [435–438]. However, local quinolone-treatment options based on local surveillance data may be reasonable, because of the geographical variations in resistance. All regimens used to treat PID should cover both, N. gonorrhoeae and C. trachomatis, so that the use of fluoroquinolones in this indication is limited, too [399]. In case parenteral β-lactam therapy is not feasible, oral use of fluoroquinolones with or without metronidazole is recommended provided treatment is based on results of antimicrobial susceptibility testing [399].

3.6. Traveller’s Diarrhea. Enterotoxigenic and enterogaegregative E. coli (ETEC and EAEC) are the major causes of bacterial traveler’s diarrhea causing up to 80% of acute cases; Shigella spp., Salmonella spp., and Campylobacter spp., as well as viruses and protozoa cause the remainder 20% of cases. Although widely present, the bacterial pathogens show seasonal as well as geographic occurrence patterns [457–460].

In the early days of fluoroquinolone treatment of gastrointestinal infections, ciprofloxacin and other fluoroquinolones were found to be highly active in vitro and clinically effective in the treatment of traveler’s diarrhea [461, 462]. However, a study performed during 1997 indicated that the MIC_{90}-values of ciprofloxacin and levofloxacin for enteropathogens collected in India, Jamaica, Mexico, and Kenya were as low as 0.125 mg/L and 0.25 mg/L; however, the individual MICs ranged from <0.0156 to 256 mg/L, thus, indicating that fluoroquinolone-resistant strains have emerged already [460]. Another study assessing the evolution of antimicrobial resistance in EAEC and ETEC causing diarrhea in patients who had traveled to different developing countries, comparing two periods of time, 1994–1997 and 2001–2004 revealed that a statistically significant increase in resistance (P<0.01) was observed for nalidixic acid and ciprofloxacin. Mutations in the gyrA gene were found in all nalidixic acid-resistant isolates, whereas mutation(s) in both gyrA and parC genes were found in the ciprofloxacin-resistant isolates. The prevalence of quinolone-resistant EAEC and ETEC was high among the isolates from patients who had travelled to North Africa (50% of EAEC and 43% of ETEC were resistant to quinolones) and among the isolates from patients who had traveled to the Indian subcontinent (66% of EAEC and 28% to 64% of ETEC resistant to quinolones). In addition, 33% of the ETEC strains from patients traveling to South-east Asia were also quinolone resistant [463–465]. Results for strains isolated from travelers to India [464], Mexico, Guatemala, India [465], and Ghana [466] confirm that fluoroquinolone resistance increased significantly during the past decade.

Recently, ESBL-producing EAEC were isolated from patients who had traveled to India [467]. Out of 51 EAEC isolates five CTX-M-15 producers were identified which were resistant to fluoroquinolones, too. Three of these five isolates belonged to the same clonal type. ESBL-producing diarrheagenic E. coli strains were isolated from children under five years of age in Nicaragua; the ciprofloxacin-MICs ranged up to 8 mg/L [468]. Diarrheagenic E. coli, in which, however, ESBL production has not been specified, were isolated from children in Brazil [469] and Vietnam [470]. The isolation of ESBL-producing diarrheagenic pathogens from children suggests that such strains being frequently multidrug-resistant are widespread in the community.

A comparison of the MIC_{90} values of ciprofloxacin for strains isolated in 1997 and 2006–2008 revealed that the susceptibilities of C. jejuni, Salmonella spp., and Shigella spp. remained unchanged, ranging from 0.06 to 0.125 [465]. However, nalidixic acid and ciprofloxacin are frequently used in several parts of the world for empirical treatment of typhoid fever and other enteric infections, so
that nalidixic acid-resistance was frequent in the 1990s already; some of the nalidixic acid-resistant strains isolated in India, Jamaica, Mexico, and Kenya were cross-resistant to ciprofloxacin [465]. Resistance to fluoroquinolones increased in enteropathogens other than E. coli over the past years causing problems in all regions of the world, including the USA and Europe [471, 472]. However, fluoroquinolone resistance differed by race, ethnicity, age, travel, and species. Only 0.5% of Shigella spp. strains isolated in the USA were ciprofloxacin resistant [473]; likewise, none of the Salmonella spp. and Shigella spp. strains isolated from children under five years with diarrhea in rural Mozambique were resistant to ciprofloxacin [474]. On the other hand, nalidixic acid resistance in Shigella spp. and Salmonella spp. strains examined in Teheran, Iran, increased from 9.2% in 2001 to 42.3% in 2005 [475], and ofloxacin-resistant Campylobacter spp. strains collected over a 11 year period in Pakistan increased from 0% in 1992 to 23% in 2002 [476]. In the UK, an increase of ciprofloxacin-resistant Campylobacter spp. from 7% in 1995 to 37.5% in 2008 was reported [477] and 80.5% of the Campylobacter spp. strains isolated in five different Portuguese cities over a five year period from 2003 to 2007 were ciprofloxacin-resistant [478]. Plasmid-mediated quinolone resistance is frequent among Salmonella spp. and Shigella spp. [42–45].

Conclusion. The fluoroquinolones have been the most effective antibiotics for the prophylaxis and treatment of bacterial travelers’ diarrhea pathogens, but increasing resistance to these agents, mainly among Campylobacter species, may limit their benefit in the future [457, 479].

4. Discussion

The emergence of resistance to fluoroquinolones in virtually all species of bacteria was recognized soon after the introduction of these compounds for clinical use [1, 480]. During the last several years, resistance to fluoroquinolones has remained very high among MRSA, P. aeruginosa and anaerobes as well as in pathogens isolated from intensive care unit-patients. More worrisome are recent reports of an overall increase in resistance to fluoroquinolones among bacteria causing community-acquired infections, such as E. coli and N. gonorrhoeae. These surveillance data demonstrate that fluoroquinolone resistance has to be associated with particular bacterial species on the one hand and patient populations on the other hand. This conclusion has been drawn by Acar and Goldstein already in 1997. These authors wrote: “The introduction of fluoroquinolones more than 10 years ago offered clinicians orally and parenterally administrable compounds with a broad spectrum of activity and therapeutic results not seen before for a wide range of infections, including complicated urinary tract infections, gastrointestinal infections, sexually transmitted diseases, respiratory tract infections, and chronic osteomyelitis. Extensive use and misuse of these compounds led to the emergence and spread of resistant strains. Widely varying percentages of resistance to fluoroquinolones have been associated with particular bacterial species, clinical settings, origins of strains, geographic locations, and local antibiotic policies” [480]. Obviously, not much has changed since then; on the contrary, resistance rates increased to alarming high rates. The continued increase in fluoroquinolone resistance rates affects patient management and necessitates a change in some current guidelines for the treatment of, for example, urinary tract infections [145–147], or even precludes the use of fluoroquinolones in the treatment of severe intra-abdominal infections [8] or sexually transmitted diseases [399, 435–438]. The consequences to be drawn are discussed indication-specifically above.

Although S. pneumoniae and H. influenzae, causing community acquired respiratory tract infections (CARTIs), remained highly susceptible to fluoroquinolones, 10- to 30% of H. influenzae and S. pneumoniae causing CARTIs harbored first-step-mutations in the quinolone resistance determining region conferring low-level fluoroquinolone resistance. These mutants pass susceptibility testing unnoticed and are primed to acquire high-level fluoroquinolone resistance rapidly, thus putting the patient at risk. Implementation of a fluoroquinolone therapy in patients harboring such first step mutants, in particular in elderly, immunocompromised patients, and patients with additional risk factors will likely result in the selection of resistance, paralleled by clinical failure.

Of major concern is the association of fluoroquinolone resistance and ESBL-production in Enterobacteriaceae. One- to two thirds of Enterobacteriaceae producing extended spectrum β-lactams were fluoroquinolone resistant, too, thus limiting the fluoroquinolone use in the treatment of community—as well as healthcare acquired urinary tract—and intra-abdominal infections as well as travelers’ diarrhea in all those geographic areas in which fluoroquinolone resistance rates and/or ESBL-production is high. The remaining ESBL-producing or plasmid-mediated quinolone resistance mechanisms harboring Enterobacteriaceae were low-level quinolone-resistant, thus, being primed to acquire high-level resistance during treatment. Furthermore, fluoroquinolones like ciprofloxacin and levofloxacin select for methicillin resistance in staphylococci. Consequently, their clinical use is limited in those indications in which staphylococci are the predominant pathogens, like skin and skin structure infections. But fluoroquinolones should be used with caution even in the treatment of infections rarely caused by staphylococci like urinary tract infections, because of the MRSA selective potential, thus causing “collateral damage” [146].

The co-selection of fluoroquinolone resistance by β-lactams or aminoglycosides, and vice versa β-lactam- or aminoglycoside resistance by fluoroquinolones demonstrates that chemically unrelated drug classes select for drug-resistant mutants and even multidrug resistant strains, so that the emergence and spread of such strains has compromised the clinical utility of diverse antibacterials.

Successful clones of resistant bacteria are often spread horizontally either due to poor hygiene, transfer of patients from one ward to another or from a hospital to a nursing home, as well as interregional migration and international population mobility. Thus, humans are mobile vectors of drug resistance [481]. Both, exposure of bacterial pathogens
to antibacterials and environmental factors have a role in the emergence and spread of resistance. Furthermore, inappropriate antibiotic policies, poor compliance, suboptimal dosing, diagnostic and laboratory error, ineffective infection control, counterfeit or altered drugs contribute to the selection of resistance. Pleiotropic factors have an impact on the fluoroquinolone resistance epidemiology; as resistance rates vary significantly between and within countries, antibiotic prescribing must be viewed against this background of diverse processes contributing to the emergence and spread of antimicrobial drug resistance.

Conflict of Interests

The author declares that he has no conflict of interest.

References


Review Article

The Dragon and the Tiger: Realities in the Control of Tuberculosis

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Received 20 February 2012; Accepted 20 April 2012

Academic Editor: Giuseppe Botta

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India and China are two Asian super-powers with developing economies carried on the shoulders of their booming populations. This growth can only be sustained by nurturing their “human resource”. However increasing reports of insufficient public health (PH) initiatives in India when compared to the aggressive PH system of China may prove to be the Achilles’ heels for India. This review compares the PH system in India and China for combating Tuberculosis (TB), the disease responsible for maximum mortality and morbidity by a single infectious agent. While China has acknowledged the disease load and thereafter has methodically improved its reporting, detection, diagnosis and treatment, India is still in denial of the imminent health risk. The Indian PH system still considers TB as a “facultative” disease for which the required control measures are already in place and functioning. Globally, India and China recorded the highest Multi-Drug Resistant TB (MDR) cases notified in 2010 (64000 and 63000, respectively). Additionally non-government sources reported extremely high proportions of MDR in India. Here we have compared the medical, social and economic approaches of the two nations towards better management and control of TB. Does India have lessons to learn from China?

1. Introduction

The Tiger and the Dragon have been pitted against each other for a while now, as both countries have asserted their right-full place on the world stage, economically and otherwise. India's economic growth, hovering around 8-9% per year, has fuelled speculation on whether and when India may catch or surpass China's over 10% growth rate [1]. While India made its presence felt by a booming “skilled-labour-middle-class” bringing the technology revolution to its door steps, China has muscled its way through an “organized-labour-lower-middle-class” propelling industry and production. The immediate byproduct of the two different growth stories is the neglected rural population and the rural population. The focus on the rural population to usher economic reforms (which was the turning point in China's economic development) has constantly attempted to achieve social objectives such as education and health care which have brought to China a holistic development. On the contrary, the “shining India” story has resulted in greater disparity between the rural and the urban [1].

The differences between India and China are, however, beyond the omnipresent economic growth. The strategic reforms (political and social) in China have placed it in a better position as compared to India. Factually, statistics reveal that life expectancy at birth in China is 73.5 years; in India it is 64.4 years. The infant mortality rate is 50 per thousand in India compared with just 17 in China. China's adult literacy rate is 94%, compared with India's 74%. Only 66% of Indian children were immunized with triple vaccine (diphtheria/pertussis/tetanus), as opposed to 97% in China. Government expenditure on health care in China is nearly 5 times that in India [1].

The burning issue which is often downplayed is the emerging burden of MDR/XDR TB in the 2 nations. It has been more than 2 decades of running National Tuberculosis Programs (NTPs) for control of tuberculosis in both the countries. Yet, India and China rank first and second in the global burden of MDR TB [2]. While China's NTP has been termed as a model NTP for nationwide prevalence surveys, a sample vital registration system and a web-based case notification system [3], India is yet to implement any of these systems.
This review attempts to compare differences between the 2 nations in combating the single largest killer infectious disease in their territories. Simple steps like notification and surveillance, to complex issues of public-private mix, spurious drugs, and malnourishment have been addressed and the efforts on the part of the two countries towards the same have been elaborated.

2. Acknowledgment of the Problem versus the Politics of Denial

Of the 37 notifiable communicable diseases in China, TB ranks first in terms of notified cases and deaths [5]. The central government began efforts to revitalize its TB control program in 2000 with a strong political commitment to tackle TB. The concept of acceptance of the problem, identifying its requirement and the political will of TB eradication, has set China on a progressive path.

The Ministry of Health (MOH) in the Government of India (GOI) on the other hand does not have a centralized list of notifiable diseases. Each state in the country has its own list and the priorities change with the state. For Maharashtra [6] and Tamilnadu [7], TB is 12th and 11th on its priority list of 24 notifiable diseases. The culture of denial is so deep rooted that, in its first ever report on public health to the people submitted in the year 2010 [8], TB has been reported to have shown decreasing trends while several nongovernmental sources have reported MDRTB in excess of 25% [9, 10]. The under-reporting of the problem by the health system is further demonstrated by recent report of totally drug resistant (TDR) TB by a tertiary health care center in Mumbai [11]. The issue is not only adequate measures to eradicate TB, but to identify and accept that TB is a substantial problem for our country.

With this perspective, we have to mention that Indian democracy is still immature in delivering an effective, functional political system. The mechanisms for converging and coordinating across ministries, state and central governments, and peripheral health governance structures are virtually absent or weak. This is unlike China (a comparatively dictatorial society), where rules have been enforced more stringently than in India. To China’s benefit and India’s dismay, at least in the health sector and specifically TB control, the nonnegotiable government stand in China has harvested some advantage.

3. The Health Delivery System: Chinese CDC Standing Tall against Indian IDSP

3.1. Centre for Disease Control and Prevention (CDC). The CDC in China is based on the example of CDC, Atlanta, GA, USA. The first such centre in China was established in 1998 in Shanghai. This model program was the precursor to a Chinese CDC created in Beijing in January 2002 and in 28 other regional provinces [12].

The creation of these centers indicates a policy response to China’s changing disease patterns, perception of disease, and the governmental changes [12]. CDC acts as a central public health organization with integrated responsibility for both community and individual health.

The CDC carries out 4 main functions:

(i) make recommendations for public health policy and planning,
(ii) surveillance,
(iii) research on preventive medicine and health care services,
(iv) provision for training and health care services.

The performance of CDC is spoken of by the mortality percentages, which have reduced from 36.98% in 1952 to 2.31% in 2001 for the infectious diseases. Of the infectious diseases that once dominated China, 12 have been eliminated in Shanghai [12]. However, the surveillance system in place has allowed keeping a track of the rising case of syphilis, gonorrhea, and TB. This allows tracing the changing disease dynamics and thus bringing in effective policy changes, which is not possible without an active surveillance mechanism.

3.2. Integrated Disease Surveillance and Planning (IDSP). IDSP is a decentralized, state-based program launched in India by the MOH in November 2004. The main objectives of the program are (1) integrating and decentralization of surveillance activities; (2) strengthening of public health laboratories; (3) human resource development—training of state surveillance officers, district surveillance officers, rapid response team, other medical and paramedical staff; and (4) use of information technology for collection, collation, compilation, analysis, and dissemination of data [13].

The project was to achieve a national coverage in 2007 through a state wise expansion in a 3-phased manner. However, reports indicate to the contrary, and the project was running a year late in 2008 [14]. The problem lay with the fact that health and health issues are a subject of the state (so as to ease the load and amend the rules as per the requirement), but in effect it just gives more diversity. There is no common agenda, and each state ends up having its own program. This leads to variable performances of the health program in different states that are least effective in containing infectious diseases which are not bound by political and geographical boundaries.

CDC managed to achieve what it had promised—disease handling, national coverage, active surveillance—all of this in changing demographics. The objectives of IDSP are not to be criticized but its lacunae or its failures will be brought to light only when the project runs to a full scale. Integration and delivery of medical services from a common source not only brings about equality of treatment but also helps address issues like, ineffective drugs, loss of patients to private sector, and manageable disease scenarios.

4. Finance

A successful programme needs to acquire, utilize, and manage incoming funds to assess impact of resources on the population health and health systems. An unhindered yet
regulated flow of money ensures sufficient and justifiable services as demanded by the system.

The total budget for TB control in China is around USD 238 million, an approximate double of that of India. The government funds around 86% of this budget and the remaining 14% is drawn from global funds. This enables China to input a little over US $200 to treat per patient which is 4 times the cost that India spends per patient [2]. This high budgetary allocation may be an added burden to the running NTP but ensures patient adherence, treatment completion and sufficient compensation for the staff.

The total budget for TB control in India for the year 2010 is around USD 100 million which has doubled in the last 5 years India. Of this a major fraction is contributed by loans, global funds, and grants while the national contribution remains a meager 1% of the total. Along with low government contribution in expenditure for TB control, India also has the lowest budget allocated in comparison to the four high burden countries [2].

As per the 2010 statistics, India spends US $50 on treating a TB patient using DOTS and USD 4000 on an MDRTB patient. Thus with an annual budget of USD 112 million, the treatment of 1982628 TB patients (USD 99,131,400) and 131000 MDR TB patient (USD 524,000,000) would require USD 623,131,400 which is a shortfall of USD 511,131,400.

The paucity of fund allocation towards TB control brings to light the lack of seriousness of the issue to the government. It also raises questions whether the funds are sufficient to buy enough stocks of medicines a prerequisite for an efficiently running DOTS program.

The looming question over India is not just the lack of funds but the source of funds. Will the change in source from global funds but the source of funds. Will the change in source from global funds to others impact the quality of drugs (monitored by the global funds) and worsen the preexisting MDR situation in the country?

5. Surveillance System and Laboratory Capacity Building

The laboratory has always played a critical role in diagnosing TB and monitoring its treatment. In the new millennium, the strength of the laboratory network is often a direct reflection of the success of TB control programs. However, high burden countries struggle to provide good-quality microscopy, with access to culture and drug susceptibility testing (DST) being scarce to nonexistent. Under such scenarios laboratory strengthening is a high priority on the TB agenda, but till then it would be ideal to improve access to and utilization of existing diagnostics [15]. The difference between India and China in terms of their laboratory capacities is shown in Table 1. With increasing MDR trends China has maintained its pace by creating more culture and drug susceptibility testing (DST) laboratories while India is still holding on largely to traditional sputum smear microscopy. While sputum smear microscopy offers the advantage of relatively low turnaround time, it lacks sensitivity and is incapable of identifying drug resistance. It is extremely important that with changing times and demands we move forward to achieve integration of technological innovations in the public domain for faster, sensitive, and more accurate diagnosis [16].

China's case detection after implementation of DOTS in 1992 was around 30%. This was despite the fact that the treatment success rate was 85%. It was not hard to realize that most of the patients were lost to the private health sector of China. The MOH then made it mandatory to report to the CDC every diagnosed case of TB. Hospitals are required to refer all patients suspected of having TB or diagnosed with it to the local CDC for further evaluation and treatment. Every working day CDC staff members seek patients who fail to report to CDC within 3 days after being reported by accessing the central database. This new disease reporting system has enabled China to achieve 75% case detection and 85% cure rates in 2005 [17]. Due to the integration of private hospitals and centralization of TB treatment, China has been able to conduct a survey of TB prevalence and drug resistance at level of every district.

The earliest reports of China's national survey on TB date back to 1979 [18]. The surveillance took into account all 31 provinces of China. Whilst sputum smears and cultures were examined at county levels, the DST was performed at the national level. For the treatment and management of MDR and XDR TB patients, China is building steps in the areas of technical support, research, drug resistance surveillance, diagnosis, and cooperation. Important issues such as adverse drug reactions, enhanced laboratory network, mathematical models to analyze the cost effectiveness of management of drug resistant TB over the next decade, implementation of rapid diagnosis method, and cooperation from the private sector have been taken into consideration [19]. The DOTS plus was launched in 1995 and has been running full scale treating at least 5000 patients per year in Hong Kong alone [20]. Pilot projects have been rapidly initiated to shift to faster drug susceptibility assays like Hains MTBDR and MGIT liquid culture DST for second-line drug testing [16].

India on the other hand is yet to achieve its first national survey. Only 2 states, Gujarat and Maharashtra have implemented statewide community-based surveys [21], on the basis of which the RNTCP reports that the prevalence of MDR TB is not increasing in the country. It is only after the TDR scare in Mumbai [11] has the local RNTCP in conjunction with the national program coordinators decided to notify MDR-TB cases for Mumbai region. We still await notification of all TB cases—the issues primarily being integration of private and public sector, maintaining patient confidentiality, and the nonexistent notification system. The proposal is to bring in notification through DST labs; however, there is heterogeneity in the testing methods (solid Versus liquid) and most labs are nonaccredited.

### Table 1: Microbiology facilities 2010 (adapted from WHO report 2009) [2].

<table>
<thead>
<tr>
<th>Laboratories</th>
<th>India</th>
<th>China</th>
</tr>
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<tbody>
<tr>
<td>Smear (/100000 population)</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Culture (/5 million population)</td>
<td>0.1</td>
<td>3.5</td>
</tr>
<tr>
<td>DST (/10 million population)</td>
<td>0.2</td>
<td>1.2</td>
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</table>
After the initiation of DOTS plus in 2007, the RNTCP promised extensive efforts for a nationwide coverage by 2010. However, only 10 states in India have DOTS plus running at sentinel sites [22]. The issue of losing patients to the vast private sector is a huge problem in India [23]. Yet, we do not have a central reporting system and database to track TB patients and their progress. This noncentralized, unchecked business of treating TB patients is adding to the burden of MDR and XDR cases. To cater to the need of such 131,000 MDR TB cases, a total of 28 intermediate reference laboratories (IRLs) under the 4 national reference laboratories [2] are existent in the Indian system. A World Bank report states that from 2009 to 2010 a total of merely 969 cases have been started on DOTS plus treatment [24] leaving a vast majority untreated.

A national survey including an all patient database will help India to realistically measure the load and pattern of drug-resistant disease which has arisen largely due to noncompliance and through wrong categorization of TB patients [25]. Had there been a central reporting system, the health system staff need not have been dependent on patient recordings but would have evidence towards right categorization and thus correct treatment.

6. Surveillance in Correctional Institutes

More than 9.8 million people are held in penal institutions throughout the world mostly as pretrial detainees or as sentenced prisoners. Nearly half of this is contributed by United States (2.92 m), Russia (0.89 m), and China (1.57 m sentenced prisoners) [26]. India, at any given time, has nearly 0.35 m inmates [27]. The numbers of prisons in many countries have not kept pace with the increasing population in these countries leading to overcrowding. Overcrowding is a major cause or contributing factor to many of the health problems in prisons, most notably communicable diseases (such as TB) and mental health issues, including the use of psychoactive substances [28].

These prisoners who often go back to their communities after being released are carriers of the disease and thus assist transmission. It therefore becomes important to actively survey these institutions for disease burden. Since 1998, a TB surveillance system (A joint proposal by the MOH and the Ministry of Justice) has been put in place in all 24 correctional institutions of Hong Kong in addition to the statutory TB notification system. All sentenced prisoners are forced to undergo a chest X-ray, sputum smear microscopy and if necessary other specialized investigations in public hospitals. If found positive the inmate is immediately started on DOTS. The treatment is monitored by chest physicians who regularly visit all the prison clusters covering major prisons in Hong Kong [29].

The surveillance data indicates a stable trend of disease with 836 active TB cases being detected in the 7-year period. The highest numbers of cases (441/836) were diagnosed within 3 months of incarceration. This surveillance system has not only allowed for early identification of the disease but also assisted in reducing disease severity and prevention of transmission within prisons and back to the community.

In contrast, India exhibits weak attempts to identify, prioritize, and manage diseases in the prison. There have been no systematic studies examining these issues. Health-related interventions in prisons have not been scrutinized or evaluated [30]. Thus, the Ministry of Health and Family Welfare (MOHFW) has never attempted a surveillance of prisons to identify the prevalence of TB in these institutions. Independent studies such as the one from Hindalga, Belgaum, Karnataka reported 2% prevalence of TB in a central jail [31], while the study from Western Maharashtra observed 51.51% deaths (34/66) due to TB between 2001 and 2008 [32].

In an independent letter by the National Human Rights Commission (NHRC) to the Inspector General (Prisons)/Chief Secretaries of States/Administrators of Union Territories [27], the joint secretary had pointed out to the authorities that one of the sample studies highlighted that nearly 79% of deaths in judicial custody were a result of infection of tuberculosis. The NHRC pointed out towards lack of not only entry level but even periodical medical checkups by the government doctors.

The lack of a surveillance system in correctional institutes, mental health institutions, and others with a further absence of statutory TB notification systems is hampering TB control efforts in India. Due to missing surveillance data, we shall probably never learn to focus our efforts where they are most needed.

7. Malnutrition

In the early 1990s, India and China were home to more than half the preschool children in the developing world who were malnourished, as measured by being stunted or underweight. In India the incidence of stunting among children aged 0–3 years was then notably higher than in China (47 versus 32%), and underweight was three times more prevalent (52 and 17%, resp.) [33].

A few key issues addressed by the Chinese health authorities as compared to India were as follows.

(i) China pursued a successful poverty alleviation strategy along with rapid economic growth.

(ii) Effective nutrition, health, and family-planning interventions were implemented at a large scale in China.

Complementary interventions such as increasing the proportion of household consuming iodized salt increased from 51% in 1990 to 95% in 2005. India’s disturbing finding is the decline in the use of iodized salt from 49.3% (as per NFHS-2, 1998-1999) to 36.7% (RCH-2, 2002-3) [34]. China increased the coverage of piped water and improved sanitation to 72% and 65% of the population, respectively. The 2000 WHO and UNICEF global water supply and sanitation data indicates that 92% and 73% of urban Indian had access to improved water supply and sanitation, respectively. However, description of water provision in many city case studies suggested that a much smaller proportion of people had access to safe, sufficient provisions [35]. The share of mothers in China who had completed middle school increased from 32% to 57%, and the share of illiterate women fell from
The percentage of women in India who had completed middle school fell from 18% in 1983 to 10% in 2004. The adult literacy rate in India is 61% compared to China’s 91% [38].

The figures for malnutrition in children in India are at a shocking 43%. Even in sub-Saharan Africa, which most people assume to have the direst poverty statistics, the average child-malnutrition rate is 28%. In an all-front effort, China cut child malnutrition by two-thirds between 1990 and 2002. Today only 7 percent of Chinese children under age 5 are underweight [39]. This means that China reached its Millennium Development Goal (MDG) by 2002, more than a decade ahead of the target year 2015 [33].

Malnutrition is an important risk factor for the development of TB. Malnutrition profoundly affects cell-mediated immunity (CMI), and CMI is the principle host defense against TB. Changes in the movement and proliferation of T-lymphocyte subpopulations in response to specific antigens, and changes in the production of key cytokines, in the formation of organized granulomas, and in macrophage activation, have been identified as important components of the process [40].

While simple effective measures like hygienic living conditions and adequate nutrition may not bring the end of TB but will definitely ease the disease burden. A literate society can take preventive measures because they tend to be more aware and hence more cautious. The government should invest more in food, water, and social security which has historically proven to reduce TB burden in different societies [41].

8. Rural Poor

The association of TB with poverty is well established at the population and neighborhood level, usually in relation to socioeconomic disadvantage and associated ethnicity or class [42]. Both countries are fighting the poverty problem on two fronts: the urban and rural. For both China and India, poverty rates are higher in rural than in urban areas. In addition, rural areas are still home to most of the total population, and poverty is thus concentrated in rural areas [43].

China’s health system has two distinctly separate parts— rural and urban. Rural health care has three levels of provision— county, township, and village. Under the post-1979 economic reforms, rural health financing has been decentralized [42]. Though the government funds have decreased towards TB treatment in rural areas, a lot of funding from external sources has allowed China to deal with the problem of rural TB.

Two projects, the Infectious and Endemic Disease Control (IEDC) project supported by the World Bank and the MOH, were initiated in 1992 and 1993 covering 573 million people in 1208 counties. This included diagnosis and free treatment if tested positive. Projects funded by Japanese government and managed by Japan international cooperation agency and a project in Tibet and Inner Mongolia being funded by Damien Foundation, Belgium is functional [44]. Despite concerted efforts there is a vast discrepancy in the accessibility to health services in rural areas in China. This fact has been openly accepted by the MOH in China which is taking measures to improve upon the shortage and allocation of health resources in poor areas of China. Economic constraints are the major reason why rural poor in China does not access health care facilities for TB care from the government [45].

The Indian government follows the westernized hospital-based medical education and training system. The health care systems are run by the constituent states and territories in India. The federal government contributes 15% towards the total expenditure mostly through national health reforms. It is only in 2005 that the government initiated the National Rural Health Mission (NRHM) in 18 Indian states to improve basic health facilities for the rural poor. According to the 2001 census, 68.8% of Indian population lived in 640,867 villages and the remaining in urban conglomerates [46].

Access to health care centers, financial constraints, illiteracy, strong traditional/ethnic beliefs, and lack of reach of medicines in rural areas are the primary reasons for spread of TB in rural areas [47]. There is no documented evidence for the reach of RNTCP in Indian villages. It is most often through partnerships with nongovernment organisations (NGOs) (partly supported by state governments) or through community health workers (CHWs) that DOTS is practiced. The inaccessibility of medicines, lack of timely culture, and DST will steadily add to the burden of MDR TB in rural areas. There have been few reports of NGO working towards the betterment of TB patients in the villages of India. For instance Southern Health Improvement Santhi (SHIS—http://www.shisindia.org/) has been successfully working since 1982 for TB care and control. They were inducted by GOI into the RNTCP and allotted with 2 districts in West Bengal. They are the only NGO’s in India accredited to run 7 TB units for the state of West Bengal. The project is not just a cure and control program but a social reform effort which is dealing with the root cause of TB in these areas—poverty, lack of education, and awareness.

The Catholic Healthcare Network is the largest group in the NGO sector with more than 5,500 health care establishments in India. Eighty five percent of these health facilities are in remote rural and tribal areas, providing medical care to communities which have not been able to access the public health services. RNTCP has recently signed an MOU with 125 such centers to ensure DOTS coverage [22]. However, rising reports of increasing MDR TB cases in tribal population are a cause of concern. For instance, Jan Adhikar Manch, an organization working for the “saharia” tribe in the rural areas of a district of Madhya Pradesh, is reporting high number of TB cases, to the extent that the village is known as the “village of Widows” [48]. The prevalence of TB and number of smear positive cases increased in the Car Nicobar Island of India from 1986 to 2002 despite implementation of NTP. The most likely reason for the increase seems to be the absence of a district TB programme with enough efficiency to sustain the gains made from the one-time initial phase of special anti-TB measures [49].

The state of Chhattisgarh is a low-intensity internal conflict ridden Indian state where 80% of population is living in rural parts, and 32% are tribal. Nine out of 18 districts
are inhabited by tribal population. The state TB officer in his speech at the consultative workshop of the TB and poverty subworking group of the Stop TB partnership mentioned that “47% of TB cases are being missed by the state TB programme” [50].

These instances simply highlight the neglect of the government towards the rural areas and worse still the rural poor. It may be true that our health system strengthening is still in infancy and expecting such far-reaching goals is unrealistic, but partnerships with carefully selected functional NGO’s for the given area or for that matter strengthening of the quality of NRHM service delivery will have far-reaching impact on reducing the disease burden.

9. Urban Poor

Until the beginning of the 1990s, poverty in China was regarded largely as a rural phenomenon, and the rural poor were the focus of antipoverty policies [51]. However, in the 1990s, urban poverty came to be seen as a problem that potentially threatened a substantial percentage of the urban population. Unlike in the past, the government has not been able to provide the urban labor force with a job guarantee.

To respond to the increasingly urgent problem of urban poverty, several urban social assistance and protection schemes have started to emerge since 1997 covering only the urban residents, including a support program for laid-off employees like the Minimum Living Standard Scheme (MLSS) or di bao scheme, Medical Financial Assistance (MFA) to poor households, and the recently established social assistance stations targeted at rural migrants. Each city decided on its own poverty line depending upon the living standard of the city. The MLSS is mainly handled by the city government as per the decided poverty line [51].

One of the major deficiencies of the di bao program is the lack of coverage of health care. To remedy this problem, several cities have supplemented the di bao scheme with various types of ad hoc medical assistance programs [52].

These programs, although they vary across localities, in general provide a partial or full waiver for inpatient and outpatient services, or subsidies to enroll in medical insurance schemes for the population eligible for di bao [51].

In 2004, the Chinese government along with the UK Department for International Development (DFID) co-founded a national project named as the Urban Health and Poverty Project (UHPP). The project focuses on urban health reform and poverty alleviation and is also the country’s largest ongoing community health and medical aid programme [53] which covers cost of TB treatment of several patients.

India is yet to put a number to the poor surviving in urban areas of the country in the past 10 years. The survey began in June 2011 and was expected to be completed by December 2011. This is important in the context of the proposed Food Security Act and the Rajiv Awas Yojana (RAY) which aims to make cities free of slums besides better targeting of other schemes. It is not surprising that since demographic and income data are incomplete, social data is even more scarce [54].

Across the country the poverty line is decided on the basis of daily consumption and expenses per head at INR 20 (US $0.38). Policies for urban poor have been in existence since the 1950’s, but the primary objective has been urban development and housing. Unlike rural areas which have an organized 3 tier health delivery structure, the urban area has no such structure available resulting in poor health indicators of urban poor.

A new health insurance scheme for the Below Poverty Line (BPL) families in the unorganized sector was formally launched on October 1, 2007 named as Rashtriya Swasthya Bima Yojna (RSBY). There is a five-year plan for rolling out the RSBY which allows each participating state to contract 20% of their respective districts each year. On one hand, the government is launching ambitious schemes for health of urban poor, and on the other hand it is minimizing the number who can access it by deciding on a value of INR20 as the poverty line cutoff (http://www.rsbjy.in/).

India’s ambitious national programme to provide quality healthcare to the country’s urban poor—the National Urban Health Mission (NUHM) which is moribund with absence of design and approach—has been shelved for the time being and will not be launched during the present 11th five-year plan. The government proposes to launch it in the 12th five-year plan (2012–2017).

10. Spurious Drugs

Counterfeit and substandard drugs are a serious and growing problem around the world—especially in less-developed countries (LDCs) (Figure 1). There are many reasons for this, including imitation, inappropriate packaging, poor manufacturing processes, and improper conditions during transportation and storage [55].
The scale of the problem remains unclear. The World Health Organisation (WHO) estimates that counterfeit drugs constitute up to 25 per cent of the total medicine supply in LDCs. In Africa and South East Asia, more detailed sampling found that between 30 and 60 per cent of medicines were substandard [4].

While 10 and 30% of all pharmaceuticals in developing countries are counterfeit, (2006 WHO figures cited in the Organization for Economic Cooperation and Development (OECD) report), India (35%) is the biggest culprit in the spurious drugs market alongwith Egypt (7%) and China (6%).

Studies have estimated that around 700,000 malaria and tuberculosis deaths per annum are attributable to fake drugs. Drug marketing surveys indicate that 94 million dollars worth of antituberculous drugs were purchased in India in the year 2006; approximately 75% of these drugs were procured from the private sector [23]. This indicates that the quality of the drugs may or may not be as per internationally recommended and accepted standards also increasing the likelihood of MDR TB.

Another study highlighting the size and characteristics of private market in High Burden Countries (HBCs) found that while the size of India’s private drug market for TB treatment was large (capable of treating around 117% of all estimated incident TB cases), China has a medium-sized market (capable of treating around 23% of all estimated incident TB cases). While India’s private market share is largely dominated by fixed-dose combinations, China’s private drug market is predominated by loose drugs [56] (Table 2).

In separate studies, it was found that in the state of Kashmir in India the rate of MDR-TB had increased which was attributed to the fake drug—purchased over the counter over a prescription from the private practitioner. The study conducted by the states’ premier research institute Sher-e-Kashmir Institute of Medical Sciences, reported lack of activity of the anti-TB drugs procured over the counter leading to high levels of MDR in the state [57].

A study conducted by randomly sampled antimalarial, antibiotic, and antinocobacterial drugs collected from pharmacies in urban and periurban areas of Delhi and Chennai, India found that 12.6% of drugs procured were spurious [58].

WHO reports that counterfeit or poor-quality anti-TB drugs are easily available in the open market. The MOH has three options: lobby for legislation that prohibits the sale of anti-TB drugs without a doctor’s prescription; accredit doctors who are trained to treat MDR-TB, and apply to the Green Light Committee for access to quality-assured second line medication [59].

At the extreme end of this scale, India and China have introduced the death penalty for certain offences involving counterfeit drugs though so far only China has actually invoked the penalty [55].

Though both countries have offered promises to check on their spurious drug stores, both are finding it difficult to take responsibility and thus necessary action. But if India and China wish to continue with pharmaceutical growth adding to their economic growth, then the 2 countries need a disease-free generation and strict measures to curb counterfeiting of drugs.

### 11. Private Practice and TB

While China’s health services are primarily financed by out-of-pocket spending (private financing), health care providers, especially the hospital industry, are still dominated by state ownership and government control (public provision) [60]. Data collected initially revealed that private sector provision of health services in China is still small and lacks sophistication [61]. Although private practitioners are gaining roots, they are relatively rare (accounting for only 3% hospitals) in the 3 Chinese provinces of Guangdong, Shanxi, and Sichuan [62].

In a study conducted in Hong Kong, it was found that of a total of 6262 notified tuberculosis patients in 2004, 1662 (26.5%) were recruited into the study; of these, 42.6% first presented to private doctors, and 57.4% to the public sector. It was observed that doctors in the public sector tend to take a Chest X-ray (CXR) and a sputum examination more often than their private counterparts. The referral time delay from private sector varied widely with 11% referred without delay, 60% by 1 month, and 17% after 3 months. These marked differences very likely reflect different clinical practices or availability of laboratory support among the various health care sectors [63].

In another study aimed to obtain details of management by private practitioners, and in particular of the antituberculosis chemotherapy, prescribed fewer patients had a sputum examination done but had a CXR when they attended to

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**Table 2: Size and characteristics of private TB market, adapted from Wells 2011.**

<table>
<thead>
<tr>
<th>Country</th>
<th>Incident cases (2008)</th>
<th>Coverage by first line private sector drugs</th>
<th>% change in volume 2004–9</th>
<th>% of private market that is loose drugs</th>
<th>Number of manufacturers with 0.3% of private first line market share</th>
<th>Fluoroquinolone coverage of MDR-TB cases</th>
<th>Fluoroquinolone coverage of all incident cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>1,982,628</td>
<td>117%</td>
<td>–3</td>
<td>23%</td>
<td>6</td>
<td>41%</td>
<td>6.1%</td>
</tr>
<tr>
<td>China</td>
<td>1,301,322</td>
<td>23%</td>
<td>59</td>
<td>98%</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

* % of all incident cases that can be treated by first line drugs in private market (average across 4 first line drugs, assuming daily 6–8 month regimen). Data for this and other columns, unless noted, are for Q4 2008–Q3 2009.

Assuming daily dosing for 18 month regimen, and no use for drug-sensitive TB.
a private clinic. Both the sputum smear-positive (65%) and
-negative cases (71%) were told that they might have TB. When
patients recalled their prescriptions or samples, it was found
that only 19% of the cases were definitely or probably
prescribed an antituberculosis regimen although this was not
always an adequate regimen [64].

The problem with the private sector and TB is not so
much an issue with China as it is with India, for 2 primary
reasons, private health sector has less scope in China, and,
secondly, the centralized system of TB notification brings all
TB cases under the national policy.

India has a huge, unwieldy, and poorly regulated private
medical sector with an estimated 10 million registered
doctors, with a ratio of 1 doctor per 1000 population, far
in excess of the WHO guideline of 1 in 3000. Around 50%
of these are qualified and registered non-allopathic doctors
practicing alternative systems of medicine such as homeo-
pathy, Ayurveda, and Unani [23]. While it has been reported
that a majority of patients do not access any health care
systems [65], nearly 50–70% of tuberculosis patients in India
continue to prefer private healthcare. These patients are not
monitored by the RNTCP and, therefore, do not benefit from
the potential success rates offered by DOTS [23].

The increasing trend of MDR in samples, sent to the
laboratory at a reputed tertiary care private hospital, gave rise
to study to evaluate the prescription practices of the private
practitioners in Mumbai, in particular the slums of Dharavi.
It was found that the participating practitioners had never
been approached or oriented by the local TB programme.
Two independent studies highlight this situation. In 1991 a
study found that 102 private doctors practicing in a slum in
the former “Bombay” showed a lack of awareness towards
regimens for TB therapy; that is, 100 private doctors pre-
scribed 80 different regimens [66]. Nearly, two decades later
another study recorded only 6 of the 106 respondents writing
a prescription with a correct drug regimen. Here 106 doctors
prescribed 63 different drug regimens [67]. There was a
tendency to overtreat with more drugs for longer durations.
Only 3 of the 106 respondents could write an appropriate
prescription for treatment of multidrug-resistant TB. These
may be single isolated reports but the rising number of slums
in the country and an equivalent rise in the number of
private medical practitioners with no regulation from the
government is a warning in itself [68].

Originally, DOTS was primarily implemented through
NTPs. It was recognized, however, that health systems are
pluralistic and that private practitioners (often general pract-
itioners) functioning in isolation from NTPs were an impor-
tant source of care for many patients though their services
did not meet international standards [69]. This fact gave rise
to the strategy of public-private mix (PPM) DOTS.

PPM DOTS is now known as “PPM for TB Care and
Control” and is a core component of the WHO STOP
TB Strategy, entitled “Engage All Care Providers” [70].
Fifty-eight of 93 countries had PPM activities in 2008. In
China, India, Nigeria, and the Philippines, PPM contributed
to detecting more than 25% TB cases while maintaining
high treatment success rates [71]. Independently, China
has contributed through PPM (mediated via general public
hospitals) to identification of 15% of all notified tuberculous
cases countrywide whereas India has contributed to 36%
of new sputum smear-positive cases in 14 selected cities
bearing a population of 50 million [71]. These figures are
not in India’s favour because a previous report evaluating the
effect of PPM launch in India reported 57% notification of
TB cases from 14 initiative centers with a population of 20
million at the end of August 2004 [72]. This is also indicative
of the fact that despite schemes and funds in place, we cannot
ensure the functioning of the scheme because there is no
followup and insistence to pursue it stringently.

Both India and China need to reassess their stand on
PPM and take it forward. With changing mind sets and pop-
ulation inclined towards the private sector in both countr-
ies, it may be to the advantage of DOTS and the government
to implement PPM more comprehensively as a combination
approach of incentives and regulation.

12. Conclusion

The economic burden of TB in developing countries is not
just on treatment of patients but also a national income
loss by a recurrently sick population. While India continues
to live in denial of the problem, China has forged ahead
by not only decreasing TB incidence, but also generating a
task force competent in handling future health emergencies.
As per WHO, China has one of the most successful TB
control programs. The fact that has added to the success
of the NTF in China is the political will and commitment
that the Chinese government has displayed. Integration of
services, a central database for case reporting and treatment,
enhanced surveillance, use of high end techniques like Hains
MTBDR plus for identification of drug resistance, increased
laboratory, and surveillance network, procurement of funds
from every possible source and its optimum utilization has
added to the credibility of the government.

India on the other hand has to begin with the acceptance
of the menace that TB poses. The annual health reports (feel
good mirages) which are still mandates and commitments
should increasingly depict results which are free from
convoluted targets. The administration and public health
system must first acknowledge that facts and figures are not
a burden but signs of a disease burden. As India continues
to pledge its commitment, it is only wasting further time before
the disease reaches epidemic proportions. India first needs
a nation-wide surveillance to correctly identify the number
of TB-affected patients. They have to be distributed in every
category so as to identify the targets and move progressively
towards achieving them. HIV/MDR/XDR will just add to
the existing burden and make treatment more expensive, ill
managed, and cumbersome.

It is also time, that we bring all TB cases under a central
cover, which not only identifies the burden from time to
time but also makes it mandatory for private practitioners
to have the same treatment approach as the DOTS. The
poor, undernourished, and immune suppressed population
needs special attention. Like China, India needs a definite
and massive boost to its public health system through rapid
increase of human resource, infrastructure, and population
outreach. We would conclude that TB is so omnipresent that only a decadent civilization/government would want to overlook it.

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Clinical Study

In Vitro Activities of Ertapenem and Imipenem against Clinical Extended Spectrum Beta-Lactamase-Producing Enterobacteriaceae Collected in Military Teaching Hospital Mohammed V of Rabat

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Received 5 February 2012; Accepted 2 May 2012

Academic Editor: Abiola C. Senok

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Objective. To study the sensitivity level of extended spectrum beta-lactamase-producing Enterobacteriaceae to Carbapenems (Imipenem, Ertapenem) marketed in Morocco and discusses the place of Ertapenem in the treatment of extended spectrum-beta-lactamase-producing.

Materials and Methods. A retrospective study of 110 extended spectrum beta-lactamase-producing Enterobacteriaceae. Isolates obtained from blood cultures, superficial and deep pus, and catheters were conducted. The minimum inhibitory concentrations of Imipenem and Ertapenem were done by the E-test. The modified Hodge test was conducted for resistant or intermediate strains.

Results. 99.1% of isolates were susceptible to Imipenem. For Ertapenem, 4 were resistant and 4 intermediate. The modified Hodge test was positive for all 08 isolates. A minimum inhibitory concentration comparison of K. pneumoniae, E. cloacae, and E. coli for Imipenem has noted a significant difference between E. cloacae on one hand and E. coli, K. pneumoniae on the other hand (P < 0.01). No significant difference was noted for minimum inhibitory concentration of Ertapenem. Conclusion. Our results confirm in vitro effectiveness of Ertapenem against extended spectrum beta-lactamase-producing Enterobacteriaceae as reported elsewhere. However, the emergence of resistance to Carbapenems revealed by production of carbapenemases in this study confirmed a necessary bacteriological documented infection before using Ertapenem.

1. Introduction

Extended spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-EB) represent a major health problem because of their multiple resistances to antibiotics. Treatment options are limited, often using the Carbapenems, cephemycins, fosfomycin, furans, and colimycin [1–4]. The results of clinical studies suggest that Imipenem remains the primary choice of treatment for bacteria that produces ESBLs [5–9]. These results increase in overall the prescription of Imipenem, an overbill and an additional selection pressure on the ecosystem, causing and maintaining in our region the multidrug resistance Acinetobacter baumannii and Pseudomonas aeruginosa endemicity, and recently the emergence of Enterobacteriaceae carbapenem-resistant strains [10, 11]. In Morocco, there are two available Carbapenems: Imipenem (IMP) and Ertapenem (ERT). Ertapenem is the second molecule of the family on the market since 2008.

The aim of our work was to study the ESBL-EB sensitivity to Carbapenems marketed in Morocco, to discuss the impact of use of Imipenem on the emergence of resistance to Carbapenems, and the Ertapenem place’s in the ESBL-EB treatment.
2. Materials and Methods

A retrospective study was conducted between January 2009 and September 2010 in the Department of Bacteriology of the Military Teaching Hospital Mohammed V of Rabat (HMIMV). Isolates of Enterobacteriaceae with a resistance phenotype-type ESBLs from blood cultures, samples of superficial and deep pus, and catheters were included. Isolates of Enterobacteriaceae without ESBL phenotype and/or isolated from the urogenital and lung samples were excluded. Duplicates were also eliminated.

Identification of Enterobacteriaceae isolates was performed by the API 20E (BioMerieux, Marcy l’Etoile, France). The detection of ESBL phenotype was performed as recommended by the Antibiotic Committee of the Microbiology French Society (CASFM) [12]. The minimum inhibitory concentrations (MIC) of IMP and ERT were determined by the E-test according to the manufacturer’s recommendations and interpreted as recommended by the CASFM (ERT: S ≤ 0.5; R > 1; IMP S ≤ 2; R > 8). The modified Hodge test was performed for resistant strains and/or intermediate to IMP and/or ERT using the technique described by Lee et al. [13]. Quality control was performed with an Escherichia coli local wild strain identified in house. Statistical analysis was performed using the SPSS 13.0 software and the results expressed as percentages for qualitative variables and as mean ± standard deviation or median and quartiles for quantitative variables. The comparison between the MIC of the different species was performed by the Kruskal-Wallis test.

3. Results

During the study period, 384 EB were isolated of which 110 (28.6%) had an ESBL phenotype. From blood cultures, 103 EB were isolated of which 43 (41.7%) had an ESBL phenotype. From samples of pus, 239 EB were isolated of which 43 (18%) had an ESBL phenotype. From sampling catheters, 42 EB were isolated of which 24 (57.14%) had an ESBL phenotype. The distribution of ESBL-EB by species, type of infection could be more disciplinary to Ertapenem, piperacillin/tazobactam, fosfomycin, and colistin [5, 14]. In fact, in vivo and in vitro data confirms that Imipenem is the best treatment for ESBL-EB infections [6–9]. In our study, in vitro activity of the two Carbapenems marketed in Morocco was determined using E-test. Susceptibility to Imipenem was 99.1%, only one isolate was intermediate. By cons, sensitivity to Ertapenem was 92.8% with four resistant (3.6%) and four intermediate (3.6%) isolates. All intermediate or resistant isolates had a positive Hodge test, demonstrating the production of carbapenemases but the typing was not performed. Considering the very limited use of Ertapenem in our establishment the resistance rate to 7.2% is maybe the base rate of resistance to Ertapenem. The modified Hodge test was positive for 08 of intermediate and resistant isolates to ERT. The IMP’s and ERT’s MIC distributions are shown in Table 2.

The MIC results were expressed as median and quartile since their distribution does not follow a normal distribution: IMP: 0.19 μg/mL [0.125, 0.25]; ERT: 0.125 μg/mL [0.032, 0.25]. Distributions of MICs of IMP and ERT of 03 major species of ESBL-EB are represented, respectively, in Tables 3 and 4.

Comparison of MIC of the three major species (K. pneumoniae, E. cloacae, and E. coli) has noted for IMP, a statistically significant difference between E. cloacae one hand and E. coli and K. pneumoniae on the other (P < 0.01). No statistically significant difference was noted for three major species with respect to the MIC of ERT.

4. Discussion

The high prevalence of ESBL-EB, particularly in blood cultures (10% of positive blood cultures and 41.7% of EB isolated) in our hospital, is a major health problem. It concerns more K. pneumoniae than E. cloacae and E. coli but the E. cloacae infection could be more difficult to treat, because of intrinsic AmpC production. The multiresistance of ESBL-EB limits the use of antibiotics to only Imipenem and secondarily to Ertapenem, piperacillin/tazobactam, fosfomycin, and colistin [5, 14]. In our study, in vitro activity of the two Carbapenems marketed in Morocco was determined using E-test. Susceptibility to Imipenem was 99.1%, only one isolate was intermediate. By cons, sensitivity to Ertapenem was 92.8% with four resistant (3.6%) and four intermediate (3.6%) isolates. All intermediate or resistant isolates had a positive Hodge test, demonstrating the production of carbapenemases but the typing was not performed. Considering the very limited use of Ertapenem in our establishment the resistance rate to 7.2% is maybe the base rate of resistance to Ertapenem. The median of IMP MIC was 0.19 μg/mL (0.125, 0.25) and the median of ERT MIC was 0.125 μg/mL (0.032, 0.25), so 75% of MIC’s are ≤0.25 μg/mL for both molecules. The in vitro activity of Ertapenem against the ESBL-EB was less than that of Imipenem. The rates of MIC of E. cloacae isolates are higher than those of K. pneumoniae and E. coli.

### Table 1: Species and samples distribution of ESBL-EB (N = 110).

<table>
<thead>
<tr>
<th>Species (no. of isolates tested)</th>
<th>Blood</th>
<th>Pus</th>
<th>Catheter</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae (94)</td>
<td>27 (39.7)</td>
<td>21 (30.9)</td>
<td>20 (29.4)</td>
<td>68 (17.7)</td>
</tr>
<tr>
<td>E. cloacae (67)</td>
<td>9 (40.9)</td>
<td>9 (40.9)</td>
<td>4 (18.2)</td>
<td>22 (5.7)</td>
</tr>
<tr>
<td>E. coli (98)</td>
<td>6 (33.3)</td>
<td>12 (66.7)</td>
<td>0</td>
<td>18 (4.7)</td>
</tr>
<tr>
<td>P. mirabilis (49)</td>
<td>0</td>
<td>1 (100)</td>
<td>0</td>
<td>1 (0.03)</td>
</tr>
<tr>
<td>P. stuartii (4)</td>
<td>1 (100)</td>
<td>0</td>
<td>0</td>
<td>1 (0.03)</td>
</tr>
<tr>
<td>Total (384)</td>
<td>43 (39.1)</td>
<td>43 (39.1)</td>
<td>24 (21.8)</td>
<td>110 (28.6)</td>
</tr>
</tbody>
</table>
Table 2: Imipenem (IMP) and Ertapenem (ERT) MIC distribution of extended spectrum beta-lactamase-producing Enterobacteriaceae.

<table>
<thead>
<tr>
<th>MIC’S</th>
<th>% of Isolates</th>
<th>IMP</th>
<th>ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>0.9</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>0.016</td>
<td>0</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>0.023</td>
<td>0</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>0.032</td>
<td>0</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>0.038</td>
<td>0.9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.047</td>
<td>0</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>0.064</td>
<td>0.9</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>0.094</td>
<td>7.5</td>
<td>6.4</td>
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<tr>
<td>0.125</td>
<td>22.4</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>0.19</td>
<td>39.2</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>13.1</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>0.38</td>
<td>2.8</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>5.6</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>1.8</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>1.8</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
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<td>0.9</td>
<td>0</td>
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</tr>
<tr>
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<td>0.9</td>
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</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0.9</td>
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</tr>
</tbody>
</table>

Table 3: Imipenem (IMP) MICs distribution of 03 principals species of ESBL-EB.

<table>
<thead>
<tr>
<th>IMP MIC</th>
<th>% of isolates</th>
<th>K. pneumoniae</th>
<th>E. cloacae</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.032</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.064</td>
<td>7.6</td>
<td>4.8</td>
<td>11.1</td>
<td></td>
</tr>
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<td>0.094</td>
<td>27.3</td>
<td>4.8</td>
<td>27.8</td>
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</tr>
<tr>
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<td>14.3</td>
<td>50</td>
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</tr>
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<td>0.25</td>
<td>10.6</td>
<td>23.8</td>
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</tr>
<tr>
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<td>9.5</td>
<td>0</td>
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</tr>
<tr>
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<td>1.5</td>
<td>23.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
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<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Ertapenem (ERT) MICs distribution of 03 main species of ESBL-EB.

<table>
<thead>
<tr>
<th>ERT MIC</th>
<th>% of isolates</th>
<th>K. pneumoniae</th>
<th>E. cloacae</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>6</td>
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<td>0</td>
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</tr>
<tr>
<td>0.016</td>
<td>7.5</td>
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</tr>
<tr>
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<td>10.4</td>
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<td></td>
</tr>
<tr>
<td>0.032</td>
<td>10.4</td>
<td>4.8</td>
<td>0</td>
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</tr>
<tr>
<td>0.047</td>
<td>3</td>
<td>0</td>
<td>9.5</td>
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</tr>
<tr>
<td>0.064</td>
<td>6</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>4.8</td>
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<td>7.5</td>
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<tr>
<td>0.75</td>
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<tr>
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<td>4.8</td>
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</tr>
<tr>
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<td>4.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
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</tr>
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<td>0</td>
<td></td>
</tr>
</tbody>
</table>

with a statistically significant difference ($P < 0.01$). In effect, 24% of E. cloacae isolates had an MIC (ERT) range of 0.75 to 1.5, 85% MIC (IMP) <0.5 μg/mL and 71% MIC (ERT) <0.25 μg/mL. The cephalosporinases hyperproduction associated with ESBL maybe explains the increase of MIC without a production of carbapenemases. These results confirm, the literature data which indicates that Ertapenem is active against ESBL-EB like E. coli and Klebsiella, but the activity is more limited for other ESBL-EB like Enterobacter spp. However, in the presence of ESBL or high produced cephalosporinases, there is usually an increase of two to eight times in the MIC of Ertapenem [15, 16]. Despite this efficiency in vitro, the use of Ertapenem as an alternative, suffers from the poverty of clinical data with often retrospective studies [17–20]. Furthermore, this use of Ertapenem, in our region, should consider two basic elements: first, the emergence of carbapenemases producing strains revealed by our study and reported by Benouda et al. [11]. This emergence is associated, in some publications, to treatment with Ertapenem [21, 22]. Second, the prescription of Carbapenems would generate a selective pressure on bacterial ecosystem and would participate in the P. aeruginosa and A. baumannii resistant Imipenem or pan-resistant strains endemicity, and consequently, this increases the risk of reducing the antibiotic arsenal.

The question arises whether the introduction of Ertapenem will have a reducing effect on the Imipenem resistance rates of P. aeruginosa and A. baumannii, species naturally resistant to Ertapenem. Some authors report that, the use of Ertapenem may help to improve the Imipenem sensitivity of P. aeruginosa by reducing unnecessary use of the IMP and the reduction of selection pressure [23–27]. According to Livemore et al., the wise use and consistency with the recommendations of the marketing authorization does not cause high risk or an additional selection of mutant’s resistant (P. aeruginosa, A. baumannii) to Carbapenems including Imipenem [28]. A study has concluded that there was no association between the changes in the sensitivity of P. aeruginosa to Carbapenems in 25 hospitals after 9 years of using Ertapenem [29]. In light of these data, Ertapenem...
should be used only in hospitals, preferably after bacteriological documentation or first line as required by the marketing authorization, after a medico-economic evaluation and if the bacterial ecology shows a significant resistance rate of Enterobacteriaceae or if no alternative for ESBL-EB suspected infection. A reassessment is mandatory after bacteriological documentation and therapeutic de-escalation, if possible. The treatment duration should be as short as possible and dosage sufficient especially in the early phase of infection (high inoculum) [19, 20, 30]. A surveillance policy and prevention are necessary to control the emergence of multiresistant strains. This justifies the efforts to prevent the spread of carbapenemases producing strains, including strict compliance with the antibiotic treatment strategies recommendations in general and in particular the use of Carbapenems [31, 32].

5. Conclusion

Although Carbapenems available in our area (Imipenem and Ertapenem) have a good activity on extended spectrum beta-lactamase-producing Enterobacteriaceae, our study reveals the existence of strains producing carbapenemases resistant to Ertapenem. This encourages the wise use of Ertapenem as an alternative to Imipenem in specific situations and efforts to prevent the emergence of these strains and their dissemination.

Conflict of Interests

The authors declared that they have no conflict of interests.

References


**Review Article**

**Glycopeptide Resistance in Gram-Positive Cocci: A Review**

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Received 20 February 2012; Revised 16 April 2012; Accepted 30 April 2012

Academic Editor: Giuseppe Botta

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Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens in the past two decades all over the world and have seriously limited the choices available to clinicians for treating infections caused by these agents. Methicillin-resistant *Staphylococcus aureus*, perhaps the most notorious among the nosocomial pathogens, was till recently susceptible to vancomycin and the other glycopeptides. Emergence of vancomycin nonsusceptible strains of *S. aureus* has led to a worrisome scenario where the options available for treating serious infections due to these organisms are very limited and not well evaluated. Vancomycin resistance in clinically significant isolates of coagulase-negative staphylococci is also on the rise in many setups. This paper aims to highlight the genetic basis of vancomycin resistance in *Enterococcus* species and *S. aureus*. It also focuses on important considerations in detection of vancomycin resistance in these gram-positive bacteria. The problem of glycopeptide resistance in clinical isolates of coagulase-negative staphylococci and the phenomenon of vancomycin tolerance seen in some strains of *Streptococcus pneumoniae* has also been discussed. Finally, therapeutic options available and being developed against these pathogens have also found a mention.

1. Introduction

Vancomycin was the first glycopeptide antibiotic to be discovered as early as 1950 [1]. However, its toxicity profile and the availability of less toxic alternatives like the beta-lactams made its use for gram-positive infections quite rare. It was only after the large-scale emergence and spread of methicillin-resistant *S. aureus* (MRSA) strains and extensive beta-lactam resistance that this agent gained prominence.

And it was not until 30 years later that the first clinical isolates with reduced susceptibility to vancomycin were described. Vancomycin resistance was first described in isolates of *Staphylococcus epidermidis* [1]. Vancomycin resistance in enterococci was first described in Europe in the late 1980s and spread to much of the developing world. The first isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan in 1997 and had a vancomycin MIC in the intermediate susceptibility range [2].

Although primary vancomycin resistance has been described in many bacterial species like *Erysipelothrix rhusiopathiae*, *Lactococcus*, *Pediococcus*, *Lactobacillus*, and so forth, which are intrinsically resistant to the glycopeptide, the current paper focusses on the problem of acquired glycopeptide resistance in gram-positive cocci.

2. Vancomycin Resistance in Enterococci

The mechanism by which vancomycin exerts its action is by preventing the synthesis of peptidoglycan precursors of the bacterial cell wall by blocking the transglycosylation step and subsequently affecting the transpeptidation step also [3, 5]. Both the transglycosylation and transpeptidation steps are essential for bacterial cell wall cross-linking.

Vancomycin resistance in enterococci was first reported by Uttley et al. in 1988 from Great Britain [8]. Vancomycin-resistant enterococci (VRE) showing resistance to glycopeptides like vancomycin and teicoplanin have now been reported from many parts of the world and show heterogeneity, both phenotypic and genotypic [9]. There are as many as 6 recognized vancomycin-resistance phenotypes—VanA, VanB, VanC, VanD, VanE, and VanG [3, 10, 11].
vancomycin resistance have been discovered. Recently, new gene clusters encoding for vancomycin resistance and the gene clusters associated with them.

2.1. Molecular Basis of Vancomycin Resistance in Enterococci. The basic mechanism of vancomycin resistance in enterococci is the formation of peptidoglycan receptors with reduced glycopeptide affinity. This results in decreased binding of vancomycin and decreased inhibition of cell wall synthesis. Peptidoglycan precursors with decreased binding to vancomycin are responsible for this. Instead of the normally occurring peptidoglycan precursor D-alanine-D-alanine, precursors like D-ala-D-lactate or D-ala-D-serine are found on the cell wall of vancomycin-resistant strains of enterococci. D-ala-D-lactate has been found to have an affinity 1000 times less than D-ala-D-ala for vancomycin whereas D-ala-D-serine has an affinity about 6 times less than the normal cell wall precursors. It has been shown that the substitution of the terminal D-alanine of the cell wall with D-lactate results in repulsive forces in the binding pocket of the vancomycin molecule leading to a 1000-fold decrease in affinity to the antibiotic. D-ala-D-serine substitution leads to a 6-fold decrease in affinity to vancomycin because of the hydroxymethyl group of serine which is bulkier than the methyl group of alanine.

2.2. Phenotypes and Genotypes of Vancomycin-Resistant Enterococci. Table 1 depicts the various phenotypes of vancomycin resistance and the gene clusters associated with them.

VanA and VanB phenotypes of vancomycin resistance are characterized by high-level resistance to vancomycin (minimum inhibitory concentrations of 64–1000 μg/mL) which is inducible in nature. VanA type, but not VanB type, strains also show high-level resistance to the other glycopeptide, teicoplanin. The inducing factors might be the previous use of glycopeptides like vancomycin in patients or the use of drugs like avoparcin and ristocetin in poultry [3]. The increased use of a glycopeptide, avoparcin, as a growth promoter in farm animals in Europe was found to be responsible for emergence of vancomycin resistant enterococci on farms making the farm animals a potential reservoir of infection for VRE in humans [14]. Avoparcin was therefore banned from the European Union in 1997 and studies have shown an attributable reduction in vancomycin resistance in enterococci isolated from human faecal carriers after the ban [15]. The other phenotypes which show inducible vancomycin resistance are VanG and VanE where the degree of resistance is low and the minimum inhibitory concentrations range from 8 to 32 μg/mL. VanD resistance phenotype is characterized by moderate- to high-level vancomycin resistance and low-level teicoplanin resistance and this resistance is also inducible in nature [16].

The other type of glycopeptide resistance seen in enterococci is constitutive, noninducible intrinsic low-level resistance, the VanCl/C2/C3 phenotypes. VanC resistance phenotype is seen in motile species of Enterococcus like Enterococcus gallinarum and Enterococcus casseliflavus. The VanC ligase of these motile strains leads to the production of D-ala-D-serine in the terminal part of the bacterial cell wall pentapeptide.

A recently discovered new gene cluster, vanN, has also been found to confer a constitutive glycopeptide resistance phenotype [4].

2.3. Vancomycin Dependence in Enterococci. Vancomycin dependence is an interesting phenomenon seen in some
strains of Enterococcus species for which growth is seen only in the presence of vancomycin as in vancomycin containing media or in patients on vancomycin therapy. The mechanism proposed for this is that vancomycin–dependent enterococci might lack a functional D-alanine–D-alanine ligase and are probably able to synthesize cell walls only from D-alanine–D-lactate precursors which are formed only in the presence of vancomycin [17].

2.4. Genetic Basis and Regulation of Vancomycin Resistance. The vanA operon responsible for the VanA type of high-level glycopeptides resistance was first found to be carried by a transposon Tn1546. This genetic element might be carried on plasmids or might be located on chromosomes [18]. The vanA and the vanB operons have been the most extensively studied of the vancomycin-resistance gene clusters. The vanA and vanB gene clusters have three major genes, vanHAX and vanH₂BX₈. These genes encode for the following proteins essential for conferring glycopeptide resistance—a dehydrogenase (VanH/VanH₂), a ligase (VanA/VanB), and a dipeptidase (VanX/VanX₂). The dehydrogenase reduces pyruvate to D-lactate, the ligase synthesizes D-alanine–D-lactate that is responsible for glycopeptide resistance, and the dipeptidase hydrolyses D-alanine–D-alanine precursors [19].

2.5. Testing for Vancomycin Resistance in Enterococci. According to the Clinical and Laboratory Standards Institute (CLSI), following are the MIC interpretative criteria for vancomycin for enterococci [20]:

(i) susceptible ≤4 μg/ml,
(ii) intermediate 8–16 μg/ml,
(iii) resistant ≥32 μg/ml.

Vancomycin screen agar is a convenient way of screening for vancomycin resistance in busy clinical microbiology labs. Vancomycin screening agar was first described by Willey et al. [21] and incorporated in the CLSI guidelines in 1993. Vancomycin screen agar plates are prepared with brain heart infusion (BHI) agar and supplemented with 6 μg/ml of vancomycin [22].

Using BHI agar instead of Mueller–Hinton agar (MHA) as the base has been proved to have a greater sensitivity and specificity [23]. Also, it has been observed that growth is sparser on MHA, thus sometimes making the interpretation of results difficult. Using 6 μg/ml of vancomycin in BHI agar has been demonstrated to show 96–99% sensitivity and 100% specificity [24]. While looking for vancomycin resistance by vancomycin screening agar, it is important to use positive and negative controls. For quality control, E. faecalis ATCC 29212 should be tested as the negative control and E. faecalis ATCC 51299 should be tested as the positive control [25].

For studying the MIC of vancomycin in enterococci, agar microdilution with MHA as the base is recommended with the various values of MICs corresponding to sensitive, resistant, and intermediate as mentioned before [26]. E-tests are a convenient substitute to the agar dilution methods for studying MICs, but the results should be interpreted carefully.

Newer methods of detection of VRE include automated culture and identification systems and chromogenic media. In the beginning, automated systems like the Vitek and MicroScan systems had problems in detecting low-level vancomycin resistance in intrinsically resistant Enterococcus species [22]. However, newer Vitek 2 and Phoenix systems have been shown to perform quite well in detecting vancomycin resistance in these organisms. In a study by Carroll et al. which evaluated the capability of the BD Phoenix Automated Microbiology System to detect vancomycin resistant strains, all vancomycin–resistant strains were correctly identified by the system [27]. The most commonly used chromogenic selective medium for screening of GRE is the bile esculin azide agar supplemented with 6–8 μg/ml of vancomycin. Until recently, it was the most commonly available screening agar also. Highly specific chromogenic substrates have been recently used to make the CHROM ID VRE (bioMérieux) and the CHROMagar GRE (BD Diagnostics). CHROM ID has chromogens which are targeted by enzymes present specifically in E. faecalis or E. faecium. The degradation of these substrates leads to the species forming purple and blue-green colonies, respectively [28]. In one study in 2008, the sensitivity and specificity of the CHROM ID agar has been evaluated to be 96.9% and 99.4%, respectively [28].

Molecular methods which detect the resistance genes responsible for resistance or decreased susceptibility to antimicrobial agents have the advantage of being rapid. These also play a major role in the understanding of the spread and genetics of enterococcal antimicrobial resistance [29]. However, since these are highly specific methods, they do not detect antimicrobial resistance due to mechanisms which are not targeted by the testing [29]. PCR protocols to directly identify VRE from stool samples have been developed and evaluated [30]. These are very helpful for surveillance of VRE using rectal swabs and stool samples and are less time consuming. Once standardized, these are also less expensive than the traditional culture screening methods. Since there are many genotypes of glycopeptide resistance, a multiplex PCR can prove helpful to detect which of the van genotypes is present in a particular isolate. One of the earliest multiplex PCR standardized to detect some of the resistance genes was by Dutka-Malen et al. in 1995 [31]. Many modifications to the PCR protocol used by Dutka-Malen et al. have been used, both in the primer sequences used and in the DNA extraction protocols.

In a study by Patel et al. in 1997, Enterococcus colonies were directly put in the PCR mixture. This particular study also used RFLP for differentiating the resistance genotypes [32]. Depardieu et al. optimized a multiplex PCR assay for detecting the genotypes of vancomycin resistance along with the species identification of E. faecalis, E. faecium, S. aureus, and S. epidermidis [33]. Real-time PCR has also been used for rapid identification and characterization of vancomycin-resistant enterococci [34].
2.6. Antimicrobial Agents Effective against VRE and Emerging Resistance. Linezolid is a member of a class of synthetic antimicrobials, the oxazolidinones. Although it is more expensive than vancomycin, linezolid has the advantage of not requiring testing for adequate serum drug concentrations or dose adjustment in renal or hepatic failure. It is therefore a valuable drug in the hands of clinicians and can be used in situations where vancomycin use is either contraindicated or ineffective. Linezolid was licensed for clinical use in the United States in 2000. It was approved for use in United Kingdom a year later. The first isolates of Enterococcus resistant to Linezolid were reported from the United Kingdom in 2002 [35]. Two of the isolates were E. faecium; one was E. faecalis. All the isolates were from patients who had been previously treated with Linezolid. Pulsed field gel electrophoresis (PFGE) analysis of the isolates indicated that resistance developed in previously susceptible strains via point mutations in the 23SrRNA [35]. Linezolid-resistant and vancomycin-resistant enterococci have also been isolated from patients without any prior therapy with Linezolid [36].

Kainer et al. performed a case-control study during a hospital outbreak of linezolid-resistant enterococci (LRE) and tried to find out putative risk factors for acquiring this infection [37]. Important risk factors which came up in the study were culture positive for MRSA, increased hospitalization duration before index culture and duration of preceding linezolid therapy [37].

Quinupristin-Dalfopristin (Q/D) is a semisynthetic antimicrobial which is administered parenterally. It belongs to a group of agents called Streptogramins. This drug has a broad spectrum of in vitro activity against gram-positive bacteria like MRSA, coagulase-negative Staphylococci, and MDR strains of Streptococcus pneumoniae [38]. It has also been found to be very effective against vancomycin strains of E. faecium [39], although against E. faecalis, it has been found to be bacteriostatic rather than bacteriocidal [40]. In a study by Winston et al., quinupristin-dalfopristin has been found to be effective in 86% of the cases of VRE in which it was used [38]. This is a well-tolerated drug apart from arthralgias or myalgias seen in higher doses in some patients.

Resistance to Dalfopristin-Quinupristin has however already emerged. In a study done in the United States by Angulo et al. on Enterococcus isolates from poultry products and human faecal samples, Q/D-resistant E. faecium was isolated in a considerable proportion [41]. The use of virginiamycin, which is also a related streptogramin and is used in poultry as a growth promoter in Europe, has most likely led to the emergence of Q/D resistance there [41].

A study from UK by Johnson et al. also showed the emergence of Q/D resistance in a few strains of E. faecium along with most of the strains of E. faecalis, E. casseliflavus, and E. gallinarum [42].

Daptomycin is a cyclic lipopeptide with rapid bactericidal activity against a wide spectrum of gram-positive bacteria. Daptomycin has been used to treat complicated skin and soft tissue infections caused by S. aureus and also for treating enterococcal infections [43]. Large-scale in vitro studies have shown that daptomycin is effective against more than 98% of enterococci tested, irrespective of their susceptibility to other agents [43].

Resistance breakpoints for daptomycin have not been defined either by the CLSI or by the EUCAST. According to the CLSI, enterococcal isolates with MIC ≤4 μg/ml are considered sensitive to daptomycin. Disk diffusion tests for daptomycin resistance are not defined by the CLSI. For MIC testing, the recommended methods are using daptomycin E-test strips with Ca²⁺ ion (40 μg/ml) adjusted MHA and broth microdilution.

A study carried out by the daptomycin study group in India demonstrated that 100% of the VRE strains were susceptible to the agent. With regards to the VSE strains however, the potency of both daptomycin and vancomycin was comparable. 90% of the E. faecium strains tested in this study were susceptible to daptomycin [44].

Tigecycline is a broad-spectrum glycylcycline antimicrobial agent which was introduced in 2005. It is a tetracycline derivative which has in vitro activity against VRE. However, clinical data regarding the efficacy of this antibiotic is lacking [45, 46].

3. Vancomycin Resistance in S. aureus

3.1. Vancomycin Intermediate S. aureus (VISA). Increasing prevalence of MRSA infections has led to the extensive use of vancomycin for treating these conditions. Infact, vancomycin is the treatment of choice for MRSA infections. However, the overuse of this antibiotic has led to the emergence of S. aureus strains with reduced susceptibility to vancomycin. Hiramatsu et al. from Japan were the first to report a clinical strain of methicillin-resistant S. aureus with reduced susceptibility to vancomycin. This strain, named Mu50, was isolated from pus from the sternal incision site of a 4-month-old male infant with pulmonary atresia [2]. Such strains have now been reported from many other countries. Tiwari and Sen were the first to report strains of S. aureus with reduced susceptibility to vancomycin (VISA) from the Indian subcontinent. Strains of S. aureus in the intermediate range of vancomycin susceptibility have also been reported from the southern part of India [47].

The concentration of vancomycin required to inhibit most strains of S. aureus ranges from 0.5 to 2 μg/ml. According to the current guidelines given by the Clinical Laboratory Standards Institute (CLSI), S. aureus isolates with vancomycin MICs between 4 and 8 μg/ml are classified as vancomycin intermediate and those with MIC ≥16 μg/ml as vancomycin resistant. These MIC cut-offs are different from those for coagulase negative staphylococci and enterococci where isolates with vancomycin MIC ≥32 μg/ml are classified as vancomycin resistant [20]. Apart from vancomycin intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA), there is one more entity, heterogenous VISA (hVISA), which is a source of some confusion. Like VISA, heterogenous VISA was first reported from Japan, from the sputum of a 64-year-old man suffering from MRSA pneumonia who had been on vancomycin therapy. The strain named Mu3 when grown on drug-free medium produced subpopulations with varying degrees of vancomycin
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Resistance [48]. hVISA has a vancomycin MIC ≤2 μg/ml by routine testing methods but has a population of cells with reduced vancomycin susceptibility (in the vancomycin intermediate range) [49].

Unlike VRSA isolates, strains of VISA or hVISA do not possess vancomycin resistant genes like vanA, vanB, or vanC. Although no mechanism has yet been conclusively established for VISA or hVISA, many mechanisms have been proposed like defects in DNA mismatch repair [50]. The acquisition of VISA phenotype is probably a multiple step event and is due to changes in the process of peptidoglycan synthesis. VISA strains have been found to synthesise excess amounts of D-alanine-D-alanine. The extra layers of cell wall precursors prevent vancomycin molecules from reaching their target sites [48].

One important difference between VRSA and heterogeneous VISA is that reduction in glycopeptide selective pressure in an environment might reduce VRSA prevalence. However, heterogeneous VISA has been found to disseminate even in the absence of glycopeptide pressure [49].

3.2. Vancomycin-Resistant S. aureus (VRSA). Ever since the discovery of vancomycin-resistant enterococcus in the late 1980s, concerns regarding the emergence of vancomycin resistance in isolates of methicillin-resistant S. aureus by transfer of plasmids was there. A fully resistant strain of vancomycin-resistant S. aureus, however, emerged only in 2002 and was first reported from the United States of America [51]. These strains, as expected, had acquired the vancomycin resistance gene cluster vanA from vancomycin-resistant enterococci. Till date, 13 cases of VRSA infection have been reported, with the majority of the cases from the United States, that too from a particular area of the country, that is, Michigan (7 of the 11 isolates from the United States were from the Michigan area) [49, 52]. The other two isolates of VRSA were from Kolkata, India, and Tehran, Iran. For 10 out of the 11 strains of VRSA reported till date, acquisition of resistance plasmids has been found to be the responsible mechanism for vancomycin resistance. For the isolate of VRSA from Tehran, the genetic basis of vancomycin resistance has not yet been established. A recently published study in 2011 from Kolkata, India, reported yet another VRSA isolate from the Indian subcontinent [53]. Vancomycin resistance has been demonstrated to be inducible in vitro for many of the VRSA strains [54]. Also, in a majority of the cases, VRE strains have been isolated along with the VRSA strains from the same patients. This goes in favour of the popular theory that the Tn1546 plasmid which carries the vanA gene cluster found in such strains is acquired from VRE [52, 55].

3.3. Special Considerations in Detecting Vancomycin Resistance in S. aureus

3.3.1. Changing CLSI Guidelines for VRSA. The susceptibility and resistance breakpoints for both minimum inhibitory concentration (MIC) and disk diffusion testing of vancomycin have been changed over time due to clinical and microbiological data which suggested that infections with strains of MRSA with vancomycin MICs more than 4 μg/ml lead to treatment failure with vancomycin. In 2006, the susceptible breakpoint for vancomycin was lowered to 2 μg/ml and the resistance breakpoint was changed to ≥16 μg/ml [56].

3.3.2. Testing for Heterogenous Vancomycin Intermediate S. aureus. Because the usual phenotypic tests cannot detect hVISA and there are no established genetic markers for vancomycin resistance in hVISA or VISA isolates, the detection of hVISA is quite difficult. The gold standard method for detection of vancomycin resistance in hVISA isolates is population analysis profile (PAP). Modifications of the PAP method where area under the curve (AUC) of the standard PAP graph is measured for the test strain and the hVISA reference strain Mu3 have been used in many studies for detecting hVISA. hVISA has been defined to have a PAP/AUC ratio of ≥0.9 [57]. Because it is not possible to carry out population analysis profile for all strains of MRSA, alternatives like the Macromethod E-test and E-test GRD have been evaluated. Varying sensitivity rates have been reported for these methods in many studies. The GRD E-test has been found to detect 71% of hVISA isolates in one study from Australia [49]. A similar study by Riederer et al. from Michigan, USA, also gave comparable results [58]. Another study from the United States gave out a much lower detection rate of only 57% by using the GRD E-test [49].

3.3.3. Resistance Phenotypes of VRSA. Depending on the degree of resistance to the glycopeptides, vancomycin, and teicoplanin, two resistance phenotypes have been defined for VRSA isolates. VRSA strains with high-level resistance to both vancomycin and teicoplanin (MIC >256 μg/ml and >32 μg/ml) are named high-level resistant VRSA (HLR VRSA). Most of the isolates till date have HLR vancomycin resistance. Only two of the VRSA isolates had a moderate level of resistance to vancomycin (MIC 32 μg/ml and 64 μg/ml) and low-level resistance to teicoplanin and were designated low-level-resistant VRSA (LLR VRSA) [52].

3.4. Treatment Options for VRSA Infections. One interesting finding that has emerged from in vitro studies and animal studies done for VRSA isolates is that vancomycin and teicoplanin show a synergistic action with β-lactams against such strains [59]. However, whether this correlates with the clinical response in patients is yet to be seen. Linezolid is one treatment option for VRSA infection which has been found to have either a synergistic or additive effect on VRSA strains when combined with ampicillin/sulbactam [60].

Daptomycin is a lipopeptide which targets the bacterial plasma membrane and causes depolarization of the membrane and loss of membrane potential. This has been used for treatment of VRSA infections, but contrary to expectations studies have found a strong positive correlation between reduced daptomycin susceptibility and vancomycin-resistance in VISA isolates [61]. Among the
other antimicrobials which show in vitro activity against vancomycin intermediate and vancomycin-resistant S. aureus are tigecycline which is a member of the tetracycline family and lipoglycopeptides like telavancin and oritavancin [49]. Newly developed cephalosporins like ceftaroline and ceftobiprole which have action against MRSA have also shown good in vitro activity against VISA and VRSA strains [62]. Whether any of these new antimicrobials emerge as viable alternatives for treatment of vancomycin nonsusceptible strains of S. aureus, however, is yet to be seen.

4. Vancomycin Resistance in Coagulase-Negative Staphylococci

Coagulase-negative staphylococci are increasingly becoming important causes of nosocomial infections. Although these are perhaps the commonest isolates from clinical samples in any diagnostic bacteriology laboratory, it is in many cases difficult to assign clinical significance to them as they are also normal commensals found on the skin surface and elsewhere. In 1987 the first clinically significant isolate of a coagulase-negative staphylococci showing resistance to vancomycin was described by Schwab et al. [63]. Since then, there have been many reports of clinically relevant isolates of coagulase negative staphylococci showing resistance to glycopeptides. Among the various species of coagulase-negative staphylococci (CoNS), Staphylococcus haemolyticus is the most common species associated with glycopeptide resistance [64]. Other species which have been associated with vancomycin resistance include S. epidermidis, Staphylococcus warneri, and Staphylococcus hominis [65]. In some studies, S. epidermidis has been found to be the commonest CoNS species associated with glycopeptide resistance [66, 67]. The prevalence of glycopeptide resistance among clinical isolates of CoNS has shown an upward trend in many studies worldwide [68].

Although the exact mechanism of glycopeptide resistance in coagulase-negative staphylococci has not yet been elucidated, glycopeptide- resistant strains of S. epidermidis and S. haemolyticus have been shown to differ considerably from glycopeptide-susceptible strains with respect to various parameters like cell wall composition and synthesis, binding to glycopeptides and even ultrastructural morphology [69]. Glycopeptide-resistant CoNS strains have been demonstrated to sequester glycopeptides like vancomycin and teicoplanin more efficiently than their glycopeptide sensitive counterparts at sites unassociated with the D-alanyl-D-alanine target [70]. Teicoplanin has been found to bind more avidly than vancomycin at these sites. Interestingly, there have been reports of strains of CoNS, especially S. haemolyticus which are resistant to teicoplanin, but vancomycin susceptible [71].

Like S. aureus, heterogenous resistance to glycopeptides has also been observed in coagulase-negative staphylococci. In some studies, population analysis profile (PAP) has been used to show the presence of populations of bacterial cells with raised glycopeptide MICs at significant frequencies (10^{-4}–10^{-5}) [72].

4.1. Detection of Vancomycin Resistance in Coagulase-Negative Staphylococci. The CLSI MIC breakpoints for coagulase-negative staphylococci differ from those for S. aureus. According to the guidelines, any coagulase-negative Staphylococcus for which vancomycin MIC is ≥32 μg/ml should be sent to a reference laboratory. For CoNS, isolates with vancomycin MIC ≤4μg/ml are considered to be sensitive to the glycopeptide, whereas those with MIC ≥32 μg/ml are classified as resistant to vancomycin [20].

Different methods have been evaluated for determining the vancomycin MIC for CoNS isolates. A few studies have demonstrated that the MICs obtained by E-test are 1-2-fold higher than those obtained by broth microdilution [73]. Automated susceptibility testing systems like Vitek 2 have also been found to give higher MIC values for vancomycin in case of CoNS isolates [74].

5. Vancomycin Tolerance in Streptococcus pneumoniae

Although vancomycin resistance is not known in Streptococcus pneumoniae, the phenomenon of vancomycin tolerance has been observed in a few strains. Though this phenomenon has also been described in some strains of S. aureus, it is with regards to S. pneumoniae that it is considered most alarming. Vancomycin tolerance has been defined as a minimum bactericidal concentration (MBC) 32-fold higher than the minimum inhibitory concentration (MIC) [75, 76]. Another definition used by workers in the field considers pneumococcal strains showing more than 1% survival after four hours of growth in the presence of vancomycin concentration more than 10 times the minimum inhibitory concentration as showing tolerance [77]. Vancomycin tolerance in S. pneumoniae has been linked to treatment failure in many cases. Vancomycin tolerant S. pneumoniae has been found to be difficult to eradicate in animal models of meningitis and clinical instances of treatment failure with vancomycin in cases of meningitis have also been reported [78]. Vancomycin tolerance in case of S. pneumoniae is important not only because it can lead to treatment failure but also because tolerance is considered a precursor phenotype to resistance [79]. The first strain of vancomycin tolerant S. pneumoniae to be isolated from the CSF of a patient with meningitis was named the Tupelo strain after the local hospital where the patient was admitted. McCullers et al. carried out studies on this strain to elucidate the mechanism behind the vancomycin tolerance. Their findings suggested a defect in the pathway which controls the phenomenon of autolysis in case of S. pneumoniae. Such a defect prevents the lysis of bacterial cell, in the presence of not only vancomycin but also other cell wall acting agents like penicillin and cephalosporins [78]. Novak et al. showed that the loss of function of one of the enzymes involved in a two-component sensor regulator system produces tolerance to vancomycin and other groups of antibiotics [79]. Experimental meningitis in rabbit models by lab mutants carrying mutations which affect the function of this enzyme (histidine kinase/phosphatase) failed to respond to vancomycin. Recent
studies have shown that vancomycin tolerance also requires the presence of a mutated capsular polysaccharide apart from the defects in the autolysin pathway [80]. Vancomycin tolerance among clinical strains of \textit{S. pneumoniae} has been reported from different parts of the world like the United States of America, Hong Kong, Columbia, and Republic of Korea [78, 81--83]. According to other studies, however, tolerance to vancomycin is not yet a major clinical problem. Many studies have failed to detect vancomycin tolerance among their strains of \textit{S. pneumoniae} [84, 85]. However, it is essential to try to detect vancomycin tolerance in \textit{S. pneumoniae} as such strains could herald the onset of resistance to vancomycin in this important pathogen.

6. Conclusion

Vancomycin has been the drug of choice for serious beta-lactam-resistant gram-positive infections for over three decades now. However, the emergence and spread of resistance to this glycopeptide as well as other glycopeptide agents like teicoplanin among clinically important gram-positive cocci like \textit{Enterococcus} species, \textit{S. aureus}, and coagulase-negative staphylococci has made it difficult to manage serious infections caused by such pathogens. Fortunately, vancomycin resistance has not yet emerged in some important pathogens like \textit{Streptococcus pyogenes}, \textit{Streptococcus agalactiae}, and \textit{Streptococcus pneumoniae}. The existence of vancomycin tolerant strains of \textit{S. pneumoniae}, however, is a cause of concern. It is important to look for alternatives to vancomycin and other glycopeptides in the treatment of serious gram-positive infections. It is also equally important to prevent the spread and emergence of glycopeptide resistance by taking proper infection control measures.

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Research Article

Bacterial Contamination of Clothes and Environmental Items in a Third-Level Hospital in Colombia

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Received 19 December 2011; Revised 21 February 2012; Accepted 29 February 2012

Academic Editor: Giuseppe Botta

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Objective. This study evaluates the bacterial contamination rate of items in the hospital setting that are in frequent contact with patients and/or physicians. By determining the bacterial species and the associated antibiotic resistance that patients are exposed to.

Methods. Hospital-based cross-sectional surveillance study of potential bacterial reservoirs. Cultures from 30 computer keyboards, 32 curtains, 40 cell phones, 35 white coats, and 22 ties were obtained.

Setting. The study was conducted in an urban academic 650-bed teaching hospital providing tertiary care to the city of Medellin, Colombia.

Results. In total, 235 bacterial isolates were obtained from 159 surfaces sampled. 98.7% of the surfaces grew positive bacterial cultures with some interesting resistance profiles.

Conclusion. There are significant opportunities to reduce patient exposure to frequently pathogenic bacteria in the hospital setting; patients are likely exposed to many bacteria through direct contact with white coats, curtains, and ties. They may be exposed to additional bacterial reservoirs indirectly through the hands of clinicians, using computer keyboards and cell phones.

1. Background

Antibiotic-resistant bacteria are implicated in an increasing amount of hospitalized patient infections worldwide. Among patients diagnosed with an infection, antibiotic resistance is associated with an increased length of hospital stay, health care costs, and patient morbidity, and mortality. Improved hand hygiene, environmental cleaning, and isolation of patients carrying pathogenic bacteria are the main methods for tackling the problem. Despite clear evidence that hygiene improves surgical outcomes, there remains considerable controversy over whether or not contaminated environmental surfaces contribute to transmission of healthcare-associated pathogens [1–8]. The risk of nosocomial infection depends on a number of factors. These include the ability of pathogens to remain viable on a surface, the rate at which contaminated surfaces are touched by patients and healthcare workers, the context in which the patient is exposed, and the levels of contamination that result in transmission to patients. Recent studies suggest that contaminated environmental surfaces may play an important role in transmission of healthcare-associated pathogens [9–23]. Clothing including white coats appears to be contaminated in the first several hours of use [24]. Other personnel effects with frequent hand contact such as pens, stethoscopes, and cell phones may have even higher levels of contamination [25].

This study demonstrates how cloth (white coats, curtains, and ties), computer keyboards, and cell phones may act as reservoirs for bacterial pathogens that may be associated with healthcare-associated infections.

2. Methods

2.1. Setting. The study was conducted in Hospital Universitario San Vicente Fundacion, an urban academic 650-bed teaching hospital providing tertiary care to the city of Medellin, Colombia. HCWs were randomly approached during routine daily patient care, and representative surfaces were randomly sampled during typical weekdays.
2.2. Sample Collection and Bacteriological Analysis. Samples were randomly collected from 30 keyboards, 32 curtains, 40 cell phones, 35 white coats, and 22 ties. At the time of the study, no active investigation was being performed for a nosocomial pathogen.

Curtains were sampled in a standardized aseptic fashion. The examiner first washed his hands and then put on a sterile surgical glove then swabbed the glove along a 25 cm² area on the lateral edge of the middle section of the curtain, because this is the area that HCWs most often contact with their hands when opening or closing the curtains. A hand imprint of the surgical glove was immediately printed onto a plate with blood agar for culture.

Ties and white coats were sampled in similar aseptic fashion by swabbing a sterile surgical glove along the whole cloth and then placing the glove onto a plate with blood agar for culture.

Keyboards and cell phones were sampled in a standardized aseptic fashion with sterile cotton-tipped applicators moisturized with Brain-Heart Infusion (BHI) liquid media. Then, the applicators were immediately used to inoculate BHI liquid transport media and sent directly to the laboratory for further procedures.

All liquid cultures were incubated for 24 hours at 35.5°C and then streaked on solid media culture plates, which were incubated for 48 hours at 35.5°C.

All isolates were Gram-stained, identification of the species and antibiotic resistance was performed by a Vitek Gram-positive and Gram-negative card (bioMérieux SA, Marcy l’Etoile, France) according to the manufacturer’s recommendations.

3. Results

In June 2011, a total of 159 samples were collected from 30 keyboards, 32 curtains, 40 cell phones, 35 white coats, and 22 ties. From all surfaces, 98.7% had bacterial contamination, and a total of 235 unique colonies were obtained.

3.1. Keyboards. From 30 keyboards sampled, a total of 39 isolations were obtained, from those, 22 (56.4%) were considered potentially clinically relevant (Table 1), highlighting bacteria as Escherichia hermannii, Methicillin-resistant S. epidermidis (MRSE), Enterococcus faecalis, Pantoea agglomerans, and Vancomycin-resistant Enterococcus faecium.

3.2. Curtains. From 32 curtains sampled, a total of 59 isolations were obtained, from those, 47 (79.6%) were considered potentially clinically relevant (Table 2), highlighting bacteria as Methicillin-resistant S. haemolyticus (MRSH), Methicillin-resistant S. cohnii (MRSC), MRSE, Methicillin-resistant S. saprophyticus (MRSS), Moraxella sp., Acinetobacter ursingii, AMP-C producer Pseudomonas oryzihabitans, Pantoea agglomerans, and Sphingomonas paucimobilis.

3.3. Cell Phones. From 40 cell phones sampled, a total of 58 isolations were obtained, from those, 51 (88%) were considered potentially clinically relevant (Table 3), highlighting bacteria as MRSH, MRSC, MRSE, Methicillin-resistant S. hominis (MRSh), Pantoea agglomerans, Acinetobacter lwofii and Sphingomonas paucimobilis.

3.4. White Coats. From 35 with coats sampled, a total of 52 isolations were obtained, from those, 39 (75%) were considered potentially clinically relevant (Table 4), highlighting bacteria as Pseudomonas oryzihabitans, MRSE, MRSH, MRSh, and Moraxella sp.

3.5. Ties. From 22 ties sampled, a total of 27 isolations were obtained, from those, 18 (66.6%) were considered

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Table 1: Distribution of bacterial isolates from keyboards.

<table>
<thead>
<tr>
<th>Type of surface</th>
<th>Number of samples per hospital area</th>
<th>Number of Isolates</th>
<th>Meticillin-resistant Staphylococcus sp.*</th>
<th>Meticillin-sensible Staphylococcus sp.*</th>
<th>Enterococcus sp.*</th>
<th>Gram-negative rods†</th>
<th>Bacillus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergency room (n = 7)</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>37.5</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Adult surgical ICU (n = 6)</td>
<td>8</td>
<td>0</td>
<td>6</td>
<td>75</td>
<td>2</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Adult medical ICU (n = 6)</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>33.3</td>
<td>1</td>
<td>11.1</td>
<td>1</td>
</tr>
<tr>
<td>Adult special care unit (n = 6)</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>22.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Internal medicine ward (n = 5)</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>39</td>
<td>2</td>
<td>14</td>
<td>35.9</td>
<td>3</td>
<td>7.7</td>
<td>3</td>
</tr>
</tbody>
</table>

* S. epidermidis. † S. epidermidis, S. aureus, S. warneri, S. haemolyticus. † One was a E. faecium resistant to vancomycin. † Pantoea agglomerans, Escherichia hermannii, Leclercia adecarboxylata.
## Table 2: Distribution of bacterial isolates from curtains.

<table>
<thead>
<tr>
<th>Type of surface</th>
<th>Number of samples per Hospital area</th>
<th>Number of Isolates</th>
<th>Meticillin-resistant Staphylococcus sp.*</th>
<th>Meticillin-sensible Staphylococcus sp.*</th>
<th>Gram-negative rods†</th>
<th>Bacillus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n%</td>
<td>n%</td>
<td>n%</td>
<td>n%</td>
</tr>
<tr>
<td>Curtains</td>
<td>(n = 32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency room</td>
<td>(n = 8)</td>
<td>13</td>
<td>4</td>
<td>30,8</td>
<td>6</td>
<td>46,1</td>
</tr>
<tr>
<td>Adult surgical</td>
<td>ICU (n = 5)</td>
<td>8</td>
<td>4</td>
<td>50</td>
<td>3</td>
<td>37,5</td>
</tr>
<tr>
<td>Adult medical</td>
<td>ICU (n = 2)</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Adult special</td>
<td>care unit (n = 6)</td>
<td>14</td>
<td>3</td>
<td>21,4</td>
<td>4</td>
<td>28,5</td>
</tr>
<tr>
<td>Internal medicine ward (n = 11)</td>
<td></td>
<td>22</td>
<td>6</td>
<td>27,2</td>
<td>7</td>
<td>31,8</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>59</td>
<td>18</td>
<td>30,5</td>
<td>21</td>
<td>35,5</td>
</tr>
</tbody>
</table>


## Table 3: Distribution of bacterial isolates from cell phones.

<table>
<thead>
<tr>
<th>Type of surface</th>
<th>Number of samples per doctor specialty</th>
<th>Number of Isolates</th>
<th>Meticillin-resistant Staphylococcus sp.*</th>
<th>Meticillin-sensible Staphylococcus sp.*</th>
<th>Gram-negative rods†</th>
<th>Bacillus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n%</td>
<td>n%</td>
<td>n%</td>
<td>n%</td>
</tr>
<tr>
<td>Cell phones</td>
<td>(n = 40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>(n = 7)</td>
<td>11</td>
<td>2</td>
<td>18,1</td>
<td>8</td>
<td>72,7</td>
</tr>
<tr>
<td>Internal medicine (n = 8)</td>
<td></td>
<td>9</td>
<td>2</td>
<td>22,2</td>
<td>5</td>
<td>55,5</td>
</tr>
<tr>
<td>Clinical resident (n = 6)</td>
<td></td>
<td>9</td>
<td>3</td>
<td>33,3</td>
<td>4</td>
<td>44,4</td>
</tr>
<tr>
<td>Surgery</td>
<td>(n = 3)</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>Surgery resident (n = 4)</td>
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<td>4</td>
<td>1</td>
<td>25</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Medical student (n = 9)</td>
<td></td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>Nurse</td>
<td>(n = 1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Nutritionist</td>
<td>(n = 2)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>58</td>
<td>11</td>
<td>19</td>
<td>35</td>
<td>60,3</td>
</tr>
</tbody>
</table>


potentially clinically relevant (Table 5), highlighting bacteria as Methicillin resistant S. aureus and MRSE.

### 4. Discussion

The prevalence of antibiotic resistant bacteria is a serious problem with important implications for hospital infection control. Some studies have found bacterial contamination in the community (of cell phones) to be nearly equivalent to hospital settings [15]. Yet antibiotic resistant bacteria remain more common in hospital settings. Although the geographic distribution of these bacteria is worldwide, the epidemiology and dissemination patterns appear to differ within and across regions [1–8]. In this study, we found an alarming number of potentially clinically relevant bacteria colonizing different surfaces, these bacterial reservoirs are a plausible source of...
Table 4: Distribution of bacterial isolates from white coats.

<table>
<thead>
<tr>
<th>Type of surface</th>
<th>Number of samples per doctor specialty</th>
<th>Number of Isolates</th>
<th>Potentially clinically relevant microorganisms</th>
<th>Potentially clinically irrelevant microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Meticillin-resistant <em>Staphylococcus</em> sp.</td>
<td>Meticillin-sensitive <em>Staphylococcus</em> sp.</td>
</tr>
<tr>
<td>White coats (n = 35)</td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>General (n = 4)</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Internal medicine (n = 12)</td>
<td>19</td>
<td>1</td>
<td>5,2</td>
<td>11</td>
</tr>
<tr>
<td>Clinical resident (n = 7)</td>
<td>7</td>
<td>2</td>
<td>28,5</td>
<td>4</td>
</tr>
<tr>
<td>Surgery (n = 5)</td>
<td>7</td>
<td>1</td>
<td>14,2</td>
<td>5</td>
</tr>
<tr>
<td>Surgery resident (n = 4)</td>
<td>5</td>
<td>1</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Medical student (n = 2)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Nutritionist (n = 1)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Overall</td>
<td>52</td>
<td>5</td>
<td>9,6</td>
<td>30</td>
</tr>
</tbody>
</table>


Table 5: Distribution of bacterial isolates from ties.

<table>
<thead>
<tr>
<th>Type of surface</th>
<th>Number of samples per doctor specialty</th>
<th>Number of Isolates</th>
<th>Potentially clinically relevant microorganisms</th>
<th>Potentially clinically irrelevant microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Meticillin-resistant <em>Staphylococcus</em> sp.</td>
<td>Meticillin-sensitive <em>Staphylococcus</em> sp.</td>
</tr>
<tr>
<td>Ties (n = 22)</td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>General (n = 4)</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Internal medicine (n = 10)</td>
<td>13</td>
<td>2</td>
<td>15,3</td>
<td>6</td>
</tr>
<tr>
<td>Surgery (n = 6)</td>
<td>6</td>
<td>1</td>
<td>16,6</td>
<td>4</td>
</tr>
<tr>
<td>Medical student (n = 2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Overall</td>
<td>27</td>
<td>3</td>
<td>11,1</td>
<td>15</td>
</tr>
</tbody>
</table>

* S. aureus, S. epidermidis. † S. epidermidis, S. hominis, S. warneri, S. aureus.

infection for patients at this tertiary level hospital and likely any other hospital worldwide.

The most important implication of our study is to highlight the role of these items as bacterial reservoirs and how HCWs should perform hand hygiene after contact with any clothes or environmental item in agreement with the recommendation of the guideline on hand hygiene in healthcare settings [1, 3]. Some other strategies to reduce the potential for transmission of pathogens from this surfaces include improved or more frequent cleaning [4, 6–8].

In contrast to previous studies on the role of environmental colonization that were performed during nosocomial pathogen outbreaks [21, 24], our study was conducted when there was no outbreak and reflects the regular daily risk of colonization or infection from hospital fomites. Bacterial contamination of items in health care settings is likely ongoing as organisms such as *Staphylococci, E. coli,* and *P. aeruginosa* survive at least 3–6 months on dried blood or cotton and as long as four weeks on other surfaces [26, 27]. Unfortunately, we did not investigate other factors in the transmission route, such as HCWs’ hand carriage and colonization of patients.

5. Conclusion

This hospital-based cross-sectional surveillance study demonstrates that a large proportion of health care workers’ clothing and personal effects were contaminated with bacterial pathogens that can result in nosocomial infections. Further research is needed to evaluate strategies to minimize the risk of patient-to-patient transmission of pathogens from other contaminated items.

Conflict of Interests

All the authors report no conflict of interests relevant to this paper.
Funding

This work was supported by Hospital Universitario San Vicente Fundacion and Universidad de Antioquia, Medellin, Colombia.

References


Research Article

Development of an Antimicrobial Stewardship Intervention Using a Model of Actionable Feedback

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Received 24 December 2011; Revised 9 February 2012; Accepted 11 February 2012

Academic Editor: Olusegun O. Soge

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We describe the development of an audit and feedback intervention to improve antibiotic prescribing in the neonatal intensive care unit (NICU) using a theoretical framework. Participants included attending physicians, neonatal fellows, pediatric residents, and nurse practitioners. The intervention was based on the “model of actionable feedback” which emphasizes that feedback should be timely, individualized, nonpunitive, and customized to be effective. We found that real-time feedback could not be provided for the parameters established in this study, as we had to collect and analyze numerous data elements to assess appropriate initiation and continuation of antibiotics and required longer intervals to examine trends in antibiotic use. We learned during focus groups that NICU clinicians strongly resisted assigning individual responsibility for antibiotic prescribing as they viewed this as a shared responsibility informed by each patient’s laboratory data and clinical course. We were able to create a non-punitive atmosphere thanks to written informed consent from NICU attendings and assurance from leadership that prescribing practices would not be used to assess job performance. We provided customized, meaningful feedback integrating input from the participants. Adapting the principles of the “model of actionable feedback” to provide feedback for antimicrobial prescribing practices proved challenging in the NICU setting.

1. Introduction

Antimicrobial stewardship interventions to promote the judicious use of antibiotics have been recommended by medical organizations and governments as critical means to reduce the burden of antimicrobial resistance, cost, and toxicity [1]. The Infectious Diseases Society of America and the Society of Healthcare Epidemiology of America recommend prospective audit of antimicrobial use with interaction and feedback to the prescriber as an evidence-based component of effective antimicrobial stewardship [2]. However, audit and feedback strategies vary widely, and implementation has yielded inconsistent results [3]. This suggests that more research is needed to determine the most effective feedback interventions, including those adapted to special patient populations. Furthermore, studies of audit and feedback interventions have rarely articulated if a theoretical approach was used to guide the choice of interventions [4].

In recognition of the need for a theoretical approach, we first framed our prescriber audit and feedback intervention using the self-regulation model developed by Bandura as a component of social cognitive theory [5]. This model of change focuses on how individuals systematically make changes in their behavior to achieve a goal, with or without the help of a coach. The individual (a) chooses a goal, (b) selects and tries some strategies to reach it, (c) self-monitors to gather data to measure success, (d) makes a judgment about his/her success, and (e) experiences an increase or decrease in confidence in his/her abilities.

Like other investigators, however, we found that this and other individual behavior change theories were not consistently successful, as they needed additional constructs
to account for organizational factors affecting behavior change [6]. One such theoretical construct is the “model of actionable feedback” developed by Hysong et al. [7]. This model describes the features of feedback strategies associated with high levels of adherence to clinical practice guidelines for common chronic diseases such as diabetes and hypertension in six Veterans Affairs Medical Centers. High performing institutions, defined as those with greater than median adherence to guidelines, employed feedback which exhibited the following process measure characteristics: (1) timeliness as monthly or more frequent feedback was associated with better performance than quarterly feedback, (2) individualization as individual rather than group or aggregated feedback was associated with better performance, (3) lack of punitiveness as staff in low-performing centers expressed concern that feedback had a punitive connotation, and (4) customizability as feedback deemed meaningful to recipients was associated with better performance.

A potential strength of the actionable feedback model is that it appears to bridge the gap between conceptual understandings and actual implementation of feedback interventions in organizational settings, particularly settings in which close cooperation between staff is essential. We used a modification of this model in a study of interdisciplinary interventions aimed to improve antibiotic prescribing in four level III NICUs affiliated with academic medical centers. While a future publication will describe the efficacy of our interventions in improving antibiotic prescribing, the goals of this paper are to describe the development and implementation of our audit and feedback intervention using a theoretical framework. We describe the challenges encountered while developing the feedback intervention for the NICU environment, the key actions undertaken to address these challenges, and early observations related to implementation of this interventions.

2. Methods

2.1. Study Design. This substudy was embedded within the Interdisciplinary NICU Antibiotic Prescribing (iNAP) Study (NINR R010821). The iNAP study goal is to improve antibiotic prescribing practices in the NICU population by studying the effects of three randomly assigned bundles of interdisciplinary interventions on antibiotic use and antimicrobial resistance. The three interdisciplinary interventions included (1) a multimodal educational program aimed to teach relevant stewardship principles from the Centers for Disease Control and Prevention (CDC) antimicrobial stewardship guidelines, (2) a computer decision support tool aimed to improve prescribing in real time for individual patients, and (3) the intervention described in this paper, that is, an audit and feedback intervention to provide NICU staff with data regarding their prescribing practices and patient outcomes. The interdisciplinary interventions were developed by the study team between September 1st 2008 and May 1st 2010.

2.2. Study Site and Subjects. The NICU at Morgan Stanley Children’s Hospital of New York-Presbyterian at Columbia University Medical Center was randomly assigned to receive the bundle of the three interdisciplinary interventions described above. The NICU has 62 beds, with approximately 1100 discharges a year, and a mean length of stay of 22 days. Approximately 9% of the infants are <1000 grams and 22% are transferred from other institutions. Providers prescribing antibiotics include 23 neonatologists, 11 NICU fellows, 19 pediatric nurse practitioners, 7 hospitalists, and 60 pediatric residents (5 per month). Written informed consent was obtained from attending physicians for participation in the study, which was approved by the Columbia University’s Institutional Review Board. Antibiotic prescribing practices were collected by trained research personnel during the baseline year (May 1st 2009 to April 30th 2010) and during the intervention year (May 1st 2010 to April 30th 2011). Data collection is ongoing for the “sustainability” year (May 1st 2011 to April 30th 2012).

2.3. Audit and Feedback Intervention. We performed several studies prior to commencement of the iNAP study to determine patterns of antimicrobial prescribing and identify inappropriate prescribing practices that would inform the audit and feedback intervention. First, we conducted a retrospective observational study of antibiotic use and conducted an ethnographic study of workflow in the four study NICUs [8, 9]. We also assessed neonatologists’ perspectives of appropriate antimicrobial prescribing practices using vignettes derived from the observational study [10]. From our preliminary work, we learned that failure to narrow antibiotic therapy and prolonged antibiotic prophylaxis are common reasons for inappropriate antibiotic use and variation in practice. We also learned that workflow in the NICU includes frequent interruptions and that decisions about antibiotic prescribing can be discontinuous. Following randomization of the study sites to the intervention bundles, we held two focus groups to assess the attending neonatologists’ perspectives about the feedback parameters and the audit and feedback process.

Based on this preparatory work, we developed 6 parameters that were utilized to audit antibiotic use (Table 1). The audit system was partially automated, but findings were reviewed by the study team to ensure accuracy. All antibiotic use was audited, and feedback related to the chosen parameters was provided to all prescribers. However, as described above, informed consent was obtained from the attending physicians, since they had ultimate responsibility for patient care.

The feedback was provided every other month during the neonatology division’s morbidity and mortality conference using power point presentations. Our study team answered questions during the presentations and afterwards in face-to-face encounters and via email and telephone.

3. Results

The development and implementation of our audit and feedback intervention in the context of the actionable feedback
model is summarized in Table 2. While the conceptual underpinnings of the theoretic model guided our approach, it was necessary to modify our feedback strategy as we encountered some challenges to implementation while adapting this model to our NICU setting. The key actions undertaken to address these challenges are described in Table 2 as well.

### 3.1. Timeliness

We found that real-time feedback could not be provided for the parameters established in this study as we had to collect and analyze numerous data elements to assess appropriate initiation and continuation of antibiotics. Secondly, we learned that because of the relatively small numbers of antibiotic courses initiated for the study...
parameters each month, longer intervals were required to examine trends in antibiotic use. Finally, we found that the NICU staff seemed unwilling to attend frequent presentations of the data and offered us limited opportunities to present the data at an existing meeting once every 2 months. Reasons given for unwillingness to attend more frequent presentations included clinical responsibilities and preexisting meeting commitments.

3.2. Individualization. We learned during focus groups that individualized feedback was not welcomed as the NICU clinicians strongly resisted assigning individual responsibility for antibiotic prescribing as they viewed this as a shared responsibility informed by each patient’s laboratory data and clinical course. The NICU attending physicians described a treatment paradigm that reflected a shared, stepwise decision-making process. It was common for one prescriber to initiate therapy while another provider discontinued, continued, or modified the regimen due to short “on-service” terms and frequent cross-coverage. Clinicians expressed concerns that the feedback would incorrectly assign responsibility for inappropriate antibiotic prescribing to the wrong provider. These concerns distracted the prescribers from the educational goals of the intervention. Therefore, consistent with the preferences articulated during focus groups, we provided aggregated antimicrobial prescribing data and thus tailored the feedback according to user preferences.

3.3. Nonpunitive. From the study onset, we took several measures to assure prescribers that data would be kept confidential, would not be provided to the leadership, and/or would not be used to appraise their performance. NICU leadership also provided the study team with a written statement agreeing with this principle. To ensure that the neonatology attending staff understood the measures being taken to protect the confidentiality of their prescribing practices, we obtained written informed consent from them. We also obtained a certificate of confidentiality from the National Institutes of Health to further protect the data. When discussing examples of antibiotic use with the group, we did not identify individual prescribers. Finally, we were careful at all stages of the study to present a positive approach to the data audits and to emphasize the goal of improving patient safety and quality.

3.4. Customizability. As previously described, we created feedback parameters that were clinically meaningful to the prescribers based on preferences expressed during focus groups. We included a neonatologist on our study team and obtained ongoing support from the NICU leadership to maximize prescribers’ receptiveness to the feedback intervention. We compared baseline parameters with the parameters during the intervention period to identify trends. We normalized the data using familiar units, that is, 100 patient-days. We also employed brief clinical vignettes without identifiers to describe examples of antibiotic use. As the novelty of the study waned and competing priorities vied for the NICU staff’s attention, as requested, we shortened our data presentations to approximately 10 minutes.

3.5. Early Observations of Impact of Intervention. After being provided with data demonstrating variations in peri-operative antimicrobial prophylaxis for infants undergoing cardiac surgery, the NICU leadership formed an interdisciplinary committee to develop prophylaxis guidelines and created a method to monitor adherence to these guidelines.

4. Discussion

To our knowledge, this is the first study to systematically examine the process of implementing an audit and feedback system for antibiotic prescribing. Our process included the use of the model of actionable feedback which provided a theoretical framework in which we could plan and assess our interventions and ultimately have potential explanations as to why our particular interventions did or did not work [4]. Furthermore, while planning this intervention, we recognized that behaviors in intensive care units are partly regulated by administrative or group decision making. Organizations may control behavior by punishing violations of rules and regulations rather than identifying and rewarding successes. Choices of improvement strategies may be constrained by organizational practices. Feedback may be slow because the data take time to process and feedback is usually provided to units rather than individuals.

We selected the model of Hysong et al. as they identified a group of factors that enabled organizational feedback to avoid these administrative constraints and function more like the individual feedback process described in the self-regulation model [7]. For example, timely feedback ensures that the prescriber can recall the details of the case including antibiotic use, be receptive to potential prescribing alternatives, and modify his/her behavior [11]. In our study, long durations of antibiotic courses, extensive data collection, and limited opportunities for data presentations did not allow real-time feedback as feedback was provided one to two months later. However, in this clinical scenario, this time interval may still be perceived as “timely” as the staff had recall for specific cases even weeks later, particularly for rare but potentially adverse outcomes such as inadequate treatment of a resistant organism. Alternatively, this delay may have reduced the effectiveness of our intervention for more routine clinical scenarios such as the duration of treatment for culture negative sepsis.

We were also unable to provide individualized feedback. Critical to individualization is accurate attribution of antibiotic decisions to prescribers. This task is difficult in critical care units and in settings with a large number of clinicians in training as antibiotic decision-making is shared or negotiated between different providers. Furthermore, during the focus groups, participants were concerned that one clinician would be “judged” for a previous clinician’s choices and such feedback could have undermined the receptiveness of the NICU team to the feedback data. Thus, we chose to present aggregate data only. In fact, aggregate data may be preferable
in a team setting such as our NICU in which a sense of collective responsibility and investment in patient safety and quality exists. Unit-level feedback may increase the receptiveness of staff and enhance a sense of communal ownership of patient safety concerns such as has been noted in strategies to improve adherence to hand hygiene and reduce central line-associated blood stream infections [12, 13].

A nonpunitive atmosphere encourages the trust of the participants and receptiveness to feedback. Several of our methods were formal (written informed consent and certificate of confidentiality at initiation of the study), which would be irrelevant in a nonresearch setting. We believe, however, that our decision to forgo individual for group feedback made it easier for the NICU staff to perceive feedback as nonpunitive. For long-term success, audit and feedback interventions not only require the support of leadership, but require a culture that encourages transparency even when adverse outcomes occur [14].

Although each prescriber could not request individual data queries, we devoted considerable effort to ensuring the customizability of our feedback intervention to make our parameters meaningful to the NICU team. Preparatory fieldwork was essential to making the intervention viable. To maximize the receptiveness of the NICU staff, we involved the NICU leadership and conducted focus groups. When presenting data at each feedback session, we reinforced study definitions for our metrics. Customizability is particularly important in a research setting where the feedback content is guided by the study protocol rather than developed by the prescribers themselves.

Our study had several limitations. The receptiveness of clinicians to specific interventions may be influenced by the leadership and social culture of the NICU. Hence, our experience may not be generalizable to other practice settings. To implement the feedback intervention, we needed the resources of the study as well as our electronic medical record system to synthesize demographic, clinical, pharmacy, and microbiological data. These resources may not be readily available in other settings.

In summary, we had only moderate success in fully incorporating the components of the model of actionable feedback in our audit and feedback intervention to improve antibiotic prescribing in the NICU. While we encountered challenges, our solutions incorporated the core principle of customizing meaningful feedback to ensure that the NICU staff would be receptive to the feedback. We believe we succeeded as evidenced by excellent participation during the feedback sessions and the crafting of guidelines for perioperative prophylaxis for cardiac surgery. A future publication will address whether our prescriber feedback intervention was successful in improving antibiotic prescribing.

5. Conclusions

Adapting the principles of the “model of actionable feedback” to provide feedback for antimicrobial prescribing practices proved challenging in the NICU setting. We found that real-time feedback on complex antibiotic prescribing was difficult. While a nonpunitive atmosphere was maintained, NICU clinicians strongly resisted assigning individual responsibility for antibiotic prescribing. We successfully provided customized, meaningful feedback integrating input from the participants.

Conflict of Interests

All authors report no conflict of interests relevant to this paper.

Acknowledgments

Funding was provided by the National Institute of Nursing Research (R01 NR010821-02). The authors acknowledge support provided by the Center for Interdisciplinary Research to Reduce Antibiotic Prescribing at the School of Nursing, Columbia University.

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