Determination of susceptibility to fosfomycin and tigecycline of Enterobacteriaceae, particularly *Escherichia coli* isolates, producing extended-spectrum β -lactamases from multiple regional Canadian hospitals

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BACKGROUND: The worldwide spread of extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae, particularly *Escherichia coli*, has significantly limited therapeutic options, especially for urinary tract infections. Although limited in their indications, fosfomycin and tigecycline are potential agents to treat infections due to ESBL-producing organisms. Although not routinely performed, susceptibility testing to both is necessary to ensure there is not an increase in resistance.

METHODS: A total of 160 isolates of ESBL-producing *E coli* were isolated from patients at multiple regional hospitals in the Interior Health Region of British Columbia from June 2009 to January 2012. Isolates were obtained from various body fluids and sites including urine (78.2%), wounds, blood, gall bladder drain and respiratory specimens. All isolates were tested using the E-test method (Etest, bioMérieux, France) for tigecycline and Kirby Bauer disk diffusion method for fosfomycin using European Committee of Antimicrobial Susceptibility Testing breakpoints for tigecycline and Clinical and Laboratory Standards Institute zone sizes for fosfomycin.

RESULTS: All 160 isolates were found to be susceptible to tigecycline, while five isolates (3.1%) were resistant to fosfomycin (four resistant, one intermediate).

CONCLUSION: Although resistance to these antibiotics has previously been reported, the present study confirmed that isolates of ESBL-producing *E coli* from the Interior Health Region of British Columbia remain highly susceptible to both tigecycline and fosfomycin.

Key Words: Antibiotic resistance; Canada; ESBL-producing Enterobacteriaceae; Fosfomycin; Regional hospitals; Southern Interior BC; Tigecycline

Extended-spectrum β -lactamase (ESBL)-producing organisms were first recognized 30 years ago and their prevalence has continued to increase worldwide (1,2). Many of these ESBL enzymes are associated with multiple resistance genes, significantly restricting traditional therapeutic options for urinary tract infection (UTI) such as amoxicillin, cephalosporins, ampicillin, aminoglycosides, trimethoprim-sulfamethoxazole

La détermination de la susceptibilité des entérobactériacées à la fosfomycine et à la tigécycline, notamment l'*Escherichia coli*, dans les isolats produisant des β -lactamases à large spectre provenant de multiples hôpitaux canadiens régionaux

HISTORIQUE : La propagation mondiale des entérobactériacées produisant des β -lactamases à large spectre (BLLS), notamment l'*Escherichia coli*, se heurte à un nombre d'options thérapeutiques très limité, particulièrement en cas d'infections urinaires. Même si leurs indications sont limitées, la fosfomycine et la tigécycline sont des agents potentiels pour traiter les infections causées par des organismes produisant des BLLS. Les tests de susceptibilité ne sont pas effectués systématiquement, mais ils sont nécessaires pour s'assurer que la résistance à ces deux agents n'augmente pas.

MÉTHODOLOGIE : Au total, 160 isolats d'*E coli* produisant des BLLS ont été isolés chez des patients provenant de multiples hôpitaux régionaux de la région de la santé Interior de la Colombie-Britannique entre juin 2009 et janvier 2012. Ces isolats provenaient de divers foyers de liquides corporels, y compris l'urine (78,2 %), les plaies, le sang, le drain de la vésicule biliaire et des spécimens respiratoires. Les chercheurs ont testé tous les isolats au moyen de la méthode E-test (Etest, bioMérieux, France) pour la tigécycline, selon le point de cassure du Comité européen des antibiogrammes, et au moyen de la méthode par diffusion des disques imprégnés de Kirby Bauer pour la fosfomycine, selon les dimensions de la zone du *Clinical and Laboratory Standards Institute*.

RÉSULTATS : Les 160 isolats étaient susceptibles à la tigécycline, tandis que cinq isolats (3,1 %) étaient résistants à la fosfomycine (quatre résistants, un intermédiaire).

CONCLUSION : Même si des cas de résistance à ces antibiotiques ont déjà été déclarés, la présente étude confirme que les isolats d'*E coli* produisant des BLLS provenant de la région de la santé Interior de la Colombie-Britannique demeurent hautement susceptibles à la fois à la tigécycline et à la fosfomycine.

and quinolones. Fosfomycin and tigecycline represent potential agents in the management of these infections. In Canada, fosfomycin is an attractive option for the management of UTI because of its single-dose therapy, low cost and good safety profile, although it is not widely available and is restricted to treatment of UTIs. Although not indicated for UTI or bacteremia, tigecycline continues to represent a

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Figure 1) A comparison between the susceptibility of extended-spectrum β -lactamase-producing Enterobacteriaceae to fosfomycin and tigecycline in our study and other locations around the world (8-12). BC British Columbia; UK United Kingdom

potential option for intra-abdominal or wound infections involving ESBL-producing *Escherichia coli*. The use of these agents is further complicated because automated susceptibility panels in Canada typically do not test for resistance to these antibiotics and, hence, off-line manual testing is required. Susceptibility testing is not typically performed because of cost and reports indicating that ESBL-producing Enterobacteriaceae are susceptible to these two antibiotics, fosfomycin and tigecycline (3). Therefore, the need to understand susceptibility patterns to these agents for more resistant organisms (ie, ESBLs) in the Interior Health Region of British Columbia (BC) is vital.

Due to the emerging threat of Enterobacteriaceae with ESBLs causing infections in hospitals worldwide, it is a benefit to regional hospitals in the BC interior and across Canada to monitor the presence of ESBL-producing Enterobacteriaceae from infected patients. Specifically, results of the present study may help determine the effectiveness of using fosfomycin or tigecycline as an alternative choice to treat infections caused by ESBL-producing bacteria.

METHODS

A total of 160 isolates of ESBL-producing *E coli* were collected from multiple regional hospitals in the Interior Health region of BC (Royal Inland Hospital, Kelowna General Hospital, Vernon Jubilee Hospital and Penticton Regional Hospital) from June 2009 to January 2012. Isolates were obtained from various body fluids and sites including urine (78.2%), wounds, blood, gall bladder drain and respiratory specimens. All isolates were tested using the E-test method (Etest, bioMérieux, France) for tigecycline and Kirby Bauer disk diffusion method for fosfomycin using European Committee of Antimicrobial Susceptibility Testing (EUCAST) breakpoints for tigecycline and Clinical and Laboratory Standards Institute (CLSI) zone sizes for fosfomycin.

All isolates were identified as ESBL-producing Enterobacteriaceae using Vitek Gram Negative identification (AST N96 panels, bioMérieux, France). Confirmatory testing for ESBL-producing *E coli* was performed using MAST discs (MASTDISCS D68C, United Kingdom).

For the susceptibility testing of fosfomycin and tigecycline, all isolates were subcultured on Mueller-Hinton plates and incubated at 35°C; the density of the culture was equalized to a 0.5 McFarland standard. All isolates were tested using the E-test method for tigecycline and Kirby Bauer disk diffusion method for fosfomycin using EUCAST breakpoints for tigecycline and CLSI zone sizes for

fosfomycin (4-6). Quality control testing was performed using *E coli* (American Type Culture Collection 25923) for the E-test and disk diffusion test.

RESULTS

All 160 isolates of ESBL-producing *E coli* were susceptible to tigecycline, while five isolates demonstrated resistance to fosfomycin (four resistant and one intermediate). These results demonstrated that overall, in Interior Health, there was 3.1% resistance to fosfomycin.

DISCUSSION

In the past 10 years, there has been a steady increase in the number of ESBL-producing Enterobacteriaceae resistant to antibiotics typically prescribed to treat UTIs (1-3,7). This has been an alarming problem because resistance is increasing at a faster rate than the development of antibiotics. More importantly, this is of great concern to clinicians because new antibiotics are needed to overcome and avoid β -lactamase activity. A study conducted in Spain in 2005 determined that of 428 *E coli* isolates producing ESBL, many from UTIs, only 0.3% of *E coli* were resistant to fosfomycin (3). ESBL-producing Enterobacteriaceae have been shown to be susceptible to tigecycline. In a study by Fritsche et al (7), 95.7% of ESBL-producing Enterobacteriaceae isolates isolated from the urinary tract, bloodstream infections, respiratory tract, skin tissue and gastrointestinal tract were susceptible to tigecycline at ≤ 2 μ g/mL. This study included isolates from Europe, North American and Latin America. Unlike tetracyclines, tigecycline is able to overcome drug-specific efflux pump acquisition and ribosomal modification (7). Our finding correlates with a number of studies published in that there is no resistance to tigecycline observed in ESBL-producing Enterobacteriaceae. There has been little resistance observed throughout the world, with the highest resistance rate observed in Spain (97.5% susceptibility) (8). There has also been little resistance to fosfomycin observed in ESBL-producing Enterobacteriaceae (8). Our data supports these reports, with 96.9% susceptibility to fosfomycin. The highest resistance rate was observed in Japan, with 73% susceptibility (8). This is a high resistance rate compared with the literature, with most studies having a recorded susceptibility rate of 90% to 100%. Our observed resistance rate fits into this range.

The increase in the prevalence of ESBL-producing Enterobacteriaceae has necessitated need for alternative therapeutic agents. Figure 1 illustrates the resistance patterns of tigecycline and fosfomycin observed in countries around the world (8-12). The susceptibility rates ranged from 73% to 100% for fosfomycin, and 97.5% to 100% for tigecycline.

CONCLUSION

ESBL-producing *E coli* remain highly susceptible to fosfomycin and tigecycline. These agents may be considered in the management of infections due to these organisms. Microbiology laboratories should consider performing susceptibility testing of these agents for multi-resistant isolates for which therapeutic options are limited.

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