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Antimicrobial susceptibility and emerging resistance determinants (bla_{CTX-M}, *rmtB*, *fosA3*) in clinical isolates from urinary tract infections in the Bolivian Chaco

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SUMMARY

Background: Bolivia is among the lowest-resourced South American countries, with very few data available on antibiotic resistance in bacterial pathogens. The phenotypic and molecular characterization of bacterial isolates responsible for urinary tract infections (UTIs) in the Bolivian Chaco are reported here.
Methods: All clinical isolates from UTIs collected in the Hospital Basico Villa Montes between June 2010 and January 2014 were analyzed ($N = 213$). Characterization included susceptibility testing, extended-spectrum beta-lactamase (ESBL) detection, identification of relevant resistance determinants (e.g., CTX-M-type ESBLs, 16S rRNA methyltransferases, glutathione S-transferases), and genotyping of CTX-M producers.
Results: Very high resistance rates were observed. Overall, the lowest susceptibility was observed for trimethoprim–sulphamethoxazole, tetracycline, nalidixic acid, amoxicillin–clavulanic acid, ciprofloxacin, and gentamicin. Of *E. coli* and *K. pneumoniae*, 11.6% were ESBL producers. Resistance to nitrofurantoin, amikacin, and fosfomycin remained low, and susceptibility to carbapenems was fully preserved. CTX-M-15 was the dominant CTX-M variant. Four *E. coli* ST131 (two being H30-Rx) were identified. Of note, isolates harbouring *rmtB* and *fosA3* were detected.
Conclusions: Bolivia is not an exception to the very high resistance burden affecting many South American countries. Optimization of alternative approaches to monitor local antibiotic resistance trends in resource-limited settings is strongly encouraged to support the implementation of effective empiric treatment guidelines.

1. Introduction

South America has long been documented as being affected by high antibiotic resistance rates.[1–5] Complex political and socio-economic factors account for this burden, and the precise quantification at the local, national, and supranational level deserves further attention.[1–4] Bolivia is one of the lowest-resourced countries of South America, and very few data on the rates and molecular epidemiology of antibiotic resistance in bacterial pathogens have been reported from this area.[1–3,5]

Since the late 1990s, cooperation and research activities addressing the phenomenon of antibiotic resistance in the Bolivian Chaco region have been performed by the present investigators, in collaboration with the Bolivian Ministry of Health.[6–13] The healthcare system of this region, which includes many rural areas and native villages, essentially relies upon small hospitals with

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limited access to clinical microbiology facilities, which prevents any systematic collection of antimicrobial susceptibility data from the routine microbiological analysis of clinical specimens. By using commensal *Escherichia coli* as an indicator for the dissemination of antibiotic resistance in enterobacteria, very high resistance rates to old antibiotics (i.e., ampicillin, trimethoprim–sulphamethoxazole, tetracycline, chloramphenicol, and nalidixic acid) and alarmingly increasing trends of resistance to newer drugs (i.e., expanded-spectrum cephalosporins and fluoroquinolones) have been observed over the last two decades.[8–13] Of note, CTX-M-type extended-spectrum beta-lactamase (ESBL) determinants were first detected in this area in the early 2000s and thereafter underwent rapid dissemination, with an evolution of the dominant CTX-M groups mirroring that observed in other South American countries (i.e., initial dissemination of CTX-M-2, subsequently replaced by CTX-M-1 and CTX-M-9 groups).[1–3,8,14–16]

In this study, data on antimicrobial susceptibility and resistance determinants of bacterial pathogens responsible for urinary tract infections (UTIs) in the Bolivian Chaco region are reported. These data were obtained by analyzing clinical isolates from UTIs collected in the laboratory of the Hospital Basico Villa Montes (one of the first clinical microbiology laboratories implemented in that region) during the first 3 years of activity.

2. Methods

2.1. Bacterial isolates

A total of 213 non-replicate clinical isolates from UTIs were included in the study. They represented all of the isolates from positive urine cultures of patients with a clinical diagnosis of UTI, submitted to the clinical microbiology laboratory of the Hospital Basico Villa Montes (Villa Montes, Tarija Department, Bolivia) since its activation in June 2010, up to January 2014. Clinical isolates from both inpatients and outpatients were included. The isolates were stored in Amies transport medium (Oxoid, Milan, Italy) at 4 °C before being transferred to Italy.

2.2. Bacterial identification and in vitro antibiotic susceptibility testing

Bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Vitek MS, bioMérieux Inc., Marcy l'Etoile, France). Antibiotic susceptibility was tested by disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[17,18] As the CLSI does not provide interpretative criteria for fosfomycin and *Enterobacteriaceae* other than *E. coli*, results for the former species were interpreted using *E. coli* breakpoints (i.e., susceptible when the inhibition zone diameter is $\geq$16 mm),[18] as also reported in other studies (Endimiani et al.[19] and references therein). Screening and confirmatory tests for ESBL detection were carried out according to CLSI standards.[18] The production of AmpC-like enzymes was suspected on the basis of resistance to cephamycins and inhibition by 3-aminophenylboronic acid.[20] In AmpC producers, the presence of ESBLs was also investigated by modified CLSI confirmatory test, as recently proposed by Poulou et al.[21]Fosfomycin non-susceptible isolates were tested for the production of glutathione S-transferases using the disk potentiation test recently developed by Nakamura et al. (based on the inhibition of glutathione S-transferases by sodium phosphonoformate).[22]

2.3. Molecular analysis techniques

The detection and characterization of $bla_{\text{CTX-M}}$ and bla_{AmpC}-like beta-lactamase genes was performed using a PCR sequencing approach, as described previously.[8,23] PCR amplification was also used for the detection and characterization of the fosfomycin resistance genes *fosA*, *fosA3*, and *fosC2* (encoding glutathione S-transferases),[24] the aminoglycoside resistance genes *armA*, *npmA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF*, *rmtG*, and *rmtH* (encoding 16S rRNA methyltransferases), and *aac(6′)Ib* (encoding aminoglycoside acetyltransferases).[25,26] The determination of *E. coli* phylogenetic groups was carried out by multiplex PCR.[27] Established PCR-based methods were used to define the *E. coli* clone B2-O25b-ST131 and its subclones H30 and H30-Rx.[28–30]

2.4. Statistical analysis

Statistical differences were determined by Chi-square test (with Yates' correction) and the unpaired *t*-test, using GraphPad Prism for Windows, version 5.0 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Patient characteristics and aetiology of UTIs

A total of 213 clinical isolates considered responsible for UTIs were collected during the study period (June 2010 to January 2014). The study started upon activation of the clinical microbiology facility. A very low number of urine samples were processed during the first 2 years of activity (due to initial difficulties in implementing the facility), while an increasing trend was observed after 2012. As a consequence, the distribution of clinical isolates over time was as follows: 2010 (7 months), $n = 10$; 2011, $n = 12$; 2012, $n = 73$; 2013, $n = 105$; 2014 (1 month), $n = 13$.

Of the 213 clinical isolates, 71 were from inpatients and 140 were from outpatients; the origin was unknown for two isolates. The overall male to female patient ratio was 40:171, with the sex of two patients unknown. Patients ranged in age from 2 months to 95 years (mean age 44 years, median age 45 years), with the age of 16 patients unknown. Inpatient and outpatient populations differed in age distribution (mean age 54 vs. 39 years, median age 55 vs. 37 years; $p = 0.0003$), while no significant difference was observed in the male to female ratio (12:59 vs. 28:111; $p = 0.57$).

Of the 213 clinical isolates, 209 (98.1%) were *Enterobacteriaceae*, three were *Pseudomonas aeruginosa* (1.4%), and one was a *Staphylococcus saprophyticus* (0.5%) (Table 1). *E. coli* represented the dominant species (79.8%), followed by *Klebsiella pneumoniae* (8.9%) (Table 1), with no differences observed between inpatients and outpatients (data not shown).

3.2. Antibiotic susceptibility

Overall, the UTI isolates collected from the Bolivian Chaco exhibited high rates of antibiotic resistance (Table 2; **Supplementary Material** Table S1).

Table 1
Aetiology of urinary tract infections in the Bolivian Chaco (2010–2014)

Species	No. of isolates	%
Escherichia coli	170	79.8
Klebsiella pneumoniae	19	8.9
Citrobacter spp	6	2.8
Enterobacter spp	5	2.3
Proteus spp	5	2.3
Morganella morganii	3	1.4
Pseudomonas aeruginosa	3	1.4
Providencia rettgeri	1	0.5
Staphylococcus saprophyticus	1	0.5