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Short communication

Prevalence of antimicrobial resistance in *Escherichia coli* and *Klebsiella* spp. in rural South India

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ABSTRACT

The emergence and dissemination of antimicrobial resistance (AMR) is an important public health problem as resistant organisms cause difficult-to-treat infections. In this study, the prevalence of AMR in *Escherichia coli* and *Klebsiella* spp. in rural South India was examined in order to aid empirical therapy. A cross-sectional prospective study was conducted during the period from January 2012 to December 2014. Routine clinical isolates of *E. coli* and *Klebsiella* spp. were tested for antimicrobial susceptibility to β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, colistin and nitrofurantoin by the Kirby–Bauer disk diffusion method and the data were documented and analyzed with one per patient analysis using WHONET software. A total of 2292 non-duplicate clinical isolates were recovered during the study period, including 1338 *E. coli* and 954 *Klebsiella* spp. The prevalence of AMR in the total isolates was as follows: amikacin, 17.3%; ertapenem, 14.4%; doripenem, 4.5%; colistin, 13.2%; and tigecycline, 4.1%. The study results indicate a high prevalence of carbapenem resistance in *Klebsiella* spp. especially from pus and urinary isolates, whilst the prevalence of aztreonam and fluoroquinolone resistance was very high in *E. coli*.

1. Introduction

Antimicrobial resistance (AMR) is the process of development of a defence/protection mechanism by micro-organisms against antimicrobial agents by means of mutation or acquisition of new genetic material. The development of resistance in microbes is neither new nor surprising [1]. However, in the recent past AMR is reported to be a growing public health problem worldwide, especially the emergence of carbapenem resistance in Enterobacteriaceae [2,3]. Although AMR has been noted in nearly all bacterial pathogens, multidrug resistance among Enterobacteriaceae represents a unique and immediate threat because of the wide range of clinical infections caused by these isolates and the rapid transmission of resistance by conjugation via plasmids [2]. According to a recent report, some strains of Enterobacteriaceae were resistant to almost all available therapeutic options, which complicates clinical management and consequently increases the mortality rate [4].

Accurate identification and documentation of resistant strains possessing public health importance is the foremost step in the control of AMR. According to a recent World Health Organization (WHO) report [5], the problem of AMR is burgeoning and neglected in India, and this could be mainly owing to the lack of valid surveillance data and stringent antibiotic policy from the country. Furthermore, to the best of our knowledge, there are no published data on the true prevalence of AMR, especially from rural South India. With increasing rates of AMR, it is essential to monitor the prevalence of drug resistance in a specific geographical region to facilitate clinicians in choosing empirical therapy, to design and implement infection control interventions and to make necessary strategies for the containment of drug resistance. Hence, this study aimed to provide valid prospective surveillance data on the prevalence of AMR in clinically significant Enterobacteriaceae members (*Escherichia coli* and *Klebsiella* spp.) that possess public health significance.

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2. Materials and methods

2.1. Study design

A cross-sectional prospective study was conducted at Government Theni Medical College and Hospital (Theni, Tamil Nadu, India), a 900-bed tertiary care teaching hospital, from January 2012 to December 2014 to measure the prevalence of AMR in *E. coli* and *Klebsiella* spp. in a rural part of South India. The study protocol was carefully reviewed and accepted by the Institutional Human Ethical Committee of the Government Theni Medical College. All clinical samples including blood, urine, pus, sputum, stool and body fluids (including cerebrospinal fluid, pleural fluid, synovial fluid and ascitic fluid) received for bacteriological investigation as part of a patient's routine clinical management were included in the study.

2.2. Isolation and identification of Escherichia coli and Klebsiella spp. from clinical specimens

Clinical specimens were collected and transported to the microbiology laboratory for culture and susceptibility investigation as per the standard procedure [6]. All clinical specimens were inoculated on blood agar (HiMedia, Mumbai, India) and MacConkey agar (HiMedia) and were incubated overnight in ambient air at 35°C for the isolation of Enterobacteriaceae. Following incubation, culture plates were examined for bacterial colonies and were scrutinized for clinical significance. Colonies of Enterobacteriaceae were presumptively identified by Gram staining as well as catalase and oxidase tests. Preliminary identification of *E. coli* and *Klebsiella* spp. was established by colony morphology and lactose fermentation on MacConkey agar. Differentiation of lactose-fermenting Enterobacteriaceae was performed by standard biochemical tests such as indole test, motility, lysine decarboxylation, H_2S production, gas production, citrate utilization and urease production [6].

2.3. Antimicrobial susceptibility testing by the Kirby–Bauer disk diffusion method

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar (HiMedia) by the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [7] using *E. coli* ATCC 25922 and ATCC 35218 as quality control strains. Clinical isolates of *E. coli* or *Klebsiella* spp. were inoculated in saline and a direct colony suspension was prepared and adjusted to a 0.5 McFarland turbidity to contain $\sim 1.5 \times 10^8$ CFU/mL. The test strain was lawn cultured on a Mueller–Hinton agar plate, then antibiotic disks were placed and the plate was incubated at 35°C for 16–18 h in ambient air. Following incubation, the diameter of the zone of inhibition was measured around each antibiotic disk and the data were stored in WHONET software v.5.6 (http://www.whonet.org). Cumulative antimicrobial susceptibility results were interpreted based on CLSI guidelines [8]. Because of the non-availability of resistance criterion to colistin, a zone of inhibition of <11 mm was recognized as non-susceptible [9] and was considered as resistant for this study. Furthermore, for tigecycline the US Food and Drug Administration (FDA) susceptibility criterion was used since CLSI breakpoints are not yet established.

2.4. Data analysis

The cumulative antimicrobial susceptibility testing data were retrieved from the computerized database (WHONET software v.5.6) and the AMR prevalence of *E. coli* and *Klebsiella* spp. was calculated with one per patient analysis using WHONET software. All analyzed data were presented as a percentage because of the high variability in the number of tests conducted with different antibiotics. Statistical analysis was performed with *Z*-test and the difference was considered significant when the *P*-value was <0.05.

3. Results

A total of 14,371 clinical specimens were tested during the study period [urine, *n* = 5317 (37.0%); pus, *n* = 3377 (23.5%); blood, *n* = 2794 (19.4%); sputum, *n* = 2239 (15.6%); stool, *n* = 329 (2.3%); body fluids, *n* = 195 (1.4%); and other specimens, *n* = 120 (0.8%)]. These specimens were collected from 12,988 patients who had attended the hospital for their ailments during the study period. Notably, 10,653 (82.0%) of the investigated patients belonged to the inpatient category and only 2002 (15.4%) were in the outpatient category; 333 (2.6%) of the patients had given a specimen in both categories.

A total of 2384 clinical isolates of *E. coli* and *Klebsiella* spp. were isolated from the above-mentioned clinical specimens, among which 1407 were *E. coli* [urine, *n* = 699 (49.7%); pus, *n* = 423 (30.1%); blood, *n* = 56 (4.0%); sputum, *n* = 97 (6.9%); stool, *n* = 114 (8.1%); body fluids, *n* = 6 (0.4%); and other specimens, *n* = 12 (0.9%)]. The remaining 977 isolates were *Klebsiella* spp. [urine, *n* = 230 (23.5%); pus, *n* = 256 (26.2%); blood, *n* = 122 (12.5%); sputum, *n* = 340 (34.8%); stool, *n* = 14 (1.4%); body fluids, *n* = 5 (0.5%); and other specimens, *n* = 10 (1.0%)], which included 849 *Klebsiella pneumoniae* and 128 *Klebsiella oxytoca*. Among the 2384 isolates, 69 *E. coli* and 23 *Klebsiella* spp. were found to be duplicate isolates from the same patient and are excluded for one per patient analysis.

The cumulative antimicrobial susceptibility data revealed a high proportion of resistance both in *E. coli* and *Klebsiella* spp. to most of the antibiotics tested (Table 1). Compared with *E. coli*, the resistance rate in *Klebsiella* spp. was lower for most of the antibiotics tested [10]. Notably, cephalosporin, monobactam and fluoroquinolone resistance was significantly lower in *Klebsiella* spp. ($P < 0.01$); however, the resistance rate to carbapenems was significantly higher compared with *E. coli* ($P < 0.01$).

The in vitro activity of different antibiotics was evaluated against *E. coli* and *Klebsiella* spp. isolated from different clinical specimens to identify the empirical therapy for different clinical infections. Interestingly, the susceptibility pattern of *E. coli* did not differ significantly between specimens; remarkably, all bloodstream isolates of *E. coli* were found to be susceptible to colistin (Table 2). In contrast to *E. coli*, the susceptibility pattern differed significantly between clinical specimens in *Klebsiella* spp. (Table 3). Notably, a high proportion of sputum isolates were susceptible to most of the tested antibiotics compared with isolates from other clinical specimens, which might have influenced the overall low resistance rate of *Klebsiella* spp. observed in this study.

4. Discussion

The current cross-sectional study documented vital information on the prevalence of AMR in *E. coli* and *Klebsiella* spp. in rural South India. To the best of our knowledge, this is the first report to document the prevalence of AMR from rural India to a wide range of antibiotics available for clinical use, including four carbapenems.

Interestingly, >90% of the *E. coli* and *Klebsiella* spp. were resistant to ampicillin. Although *Klebsiella* has been known to possess intrinsic resistance to ampicillin, the current observation