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Research Article

Carbapenem-Resistance among Some Aerobic Gram-Negative Rods Clinical Isolates in Khartoum, Sudan

Ramah A Osman[1]*, Elsadig A Haj[1], Yousof S Yousof[2] and Musa A Ali[3]

[1]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Elrazi University, Khartoum, Sudan*

[2]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, National Ribat University, Khartoum, Sudan*

[3]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan*

***Corresponding Author:** Ramah A Osman, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Elrazi University, Khartoum, Sudan.

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Abstract

Background: Antimicrobial resistance in Gram-negative bacteria is an emerging and serious global public health threat. However, enterobacteriaceae that produces carbapenemases, which are enzymes that deactivate carbapenems and most other ß-lactam antibiotics, have emerged and are increasingly being reported worldwide.

Aim: This study was conducted to assess Carbapenem-resistance among aerobic Gram-negative rods clinical isolates.

Research Methodology: One hundred eighty clinical isolates were collected from different hospitals located in Khartoum State; 60 isolates for each *E. coli, K. pneumoniae* and *Pseudomonas aeruginosa*. All clinical isolates were further tested for antimicrobial sensitivity testing by modified Kirby Bauer's method, and the carbapenem resistant isolates were tested by modified Hodge test (MHT) to confirm carbapenemase-producer.

Results: The overall resistance of selected clinical isolates to Imepenem, Ceftriaxone, Amoxicillin/Clavulinic acid, Ciprofloxacin, Gentamicin and Co-trimoxazole was 31 (17.2%), 77 (42.7%), 71(39.4%), 59 (32.7%), 48 (26.6%) and 73 (40.5%), respectively.

Higher percentages of multi-durg resistant isolates were reported in this study 74(41%). There was emerged strains of ESBL among clinical isolates 11(6.1%). Higher carbapenem-resistance was reported 31(17.2%), mostly by Carbapenemase production 20 (64.5%). So, genotyping for MHT negative results is highly recommended to determine the other resistance mechanism.

Keywords: Carbapenem; Antibiotic Resistance; Multi-Drug Resistant; ESBL; Sudan

Introduction

Resistance to antimicrobial agents among clinically important pathogens in the community and hospital settings has compromised therapy and requires constant monitoring of emerging patterns [1].

Previous reviews on this topic have cited that 50 to 60% of the more than 2 million nosocomial infections in the United States each year are caused by antimicrobial resistant bacteria [1,2]. Therefore, surveillance networks have emerged to monitor various aspects of medical practice related to infection therapy and to address the problem of antimicrobial resistance [3]. As stated by Bax., *et al*. "no ideal surveillance system" exists, but some systems may provide meaningful results that can guide empirical antimicrobial regimens and minimize the consequences of antimicrobial resistance [4].

The development of antimicrobial resistance among gram-negative pathogens has been progressive and relentless. Pathogens of particular concern include extended-spectrum β-lactamase–pro-

ducing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, and multidrug-resistant *Pseudomonas aeruginosa*. Classic agents used to treat these pathogens have become outdated. Of the few new drugs available, many have already become targets for bacterial mechanisms of resistance. Carbapenems are considered first-line agents in treating infections caused by ESBL-producing organisms [5].

Recognizing carbapenemase expression is the key to the appropriate management of infections caused by carbapenem-resistant Enterobacteriaceae. Unusually elevated MICs to carbapenems should arouse suspicion for a carbapenem-resistant isolate and preclude the use of carbapenems even if the MICs do not exceed the breakpoints for resistance. As with ESBL-producing organisms, carbapenemase-producing strains are likely to exhibit simultaneous resistance to aminoglycosides and fluoroquinolones [6].

Infections caused by gram-negative bacteria have features that are of particular concern. These organisms are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure. Furthermore, they have available to them a plethora of resistance mechanisms, often using multiple mechanisms against the same antibiotic or using a single mechanism to affect multiple antibiotics. Compounding the problem of antimicrobial-drug resistance is the immediate threat of a reduction in the discovery and development of new antibiotics. Several factors have contributed to this decline, including the increasing challenges of screening for new compounds, the high capital costs and long time required for drug development, the growing complexity of designing and performing definitive clinical trials, and the concern about reduced drug longevity due to the emergence of resistance. As a consequence, a perfect storm has been created with regard to these infections: increasing drug resistance in the absence of new drug development [7].

Pseudomonas aeruginosa is an opportunistic pathogen that causes bacteremia in immunocompromised patients and burn victims, iatrogenic urinary tract infections, and hospital-acquired pneumonia, particularly in intensive-care settings [8]. Infections are especially serious in intubated patients, with a reported mortality of up to 40 to 50% [9].

Research Methodology

- **Study design:** This is a descriptive cross-sectional study.
- **Sample size and duration:** One hundred eighty clinical isolates was collected from different hospitals located in Khartoum State during the period from August to October 2016.
- **Ethical consideration:** The approval was taken from authorities of all hospitals in Khartoum State, where the isolates were collected.

Laboratory work

- **Re-identification of clinical isolates:** All Gram-negative rods were re-identified by using standard microbiological techniques according to Cheesbrough, 2000 [10].
- **Antibiotic susceptibility testing:** Modified Kirby-Bauer disk diffusion method was used to perform sensitivity testing of Imepenem, Ceftriaxone, Amoxicillin/Clavulinic acid, Ciprofloxacin, Gentamicin and Co-trimoxazole. All phenotypic imepenem-resistant strains were confirmed by Modified Hodge test.
- **Modified Hodge test:** The Carbapenem-resistant strains were confirmed by modified Hodge test.
- **Statistical analysis:** Statistical analysis was done by using SPSS program.

Results

One hundred and eighty clinical isolates of *E. coli, Klebsiella pneumonia and Pseudomonas aeruginosa* were isolated from different clinical specimens from different hospitals in Khartoum State which collected for antimicrobial susceptibility testing against imipenem and other selected antibiotics used in clinical setting.The frequency of *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 60 (33.3%) for each isolate.

The overall resistance of selected clinical isolates to Imepenem, Ceftriaxone, Amoxicillin/Clavulinic acid, Ciprofloxacin, Gentamicin and Co-trimoxazole was 31 (17.2%), 77 (42.7%), 71(39.4%), 59 (32.7%), 48 (26.6%) and 73 (40.5%), respectively (table1).

The frequency of multi-durg resistant among *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 33 (55%), 24 (40%) and 17 (28.3%), respectively. Also some clinical isolates showed total resistance to all selected antibiotics with 18 (10%) (table 2).

Antibiotic discs	Overall resistance
Imipenem	17.2%
Ceftriaxone	42.7%
Amoclan	39.4%
Ciprofloxacin	32.7%
Gentamicin	26.6%
Co-trimoxazole	40.5%

Table 1: Overall resistance of *E. coli, Klebsiella spp. and Ps. aeruginosa* against selected antibiotics.

Organism	MDR
E. coli	33 (55%)
Klebsiella pneumoniae	24 (40%)
Pseudomonas aeruginosa	17 (28.3%)

Table 2: Multi-drug resistance in clinical isolates

MDR**;** Multi-drug resistant organisms

Of particular interest there were considerable percentages of ESBL which detected phenotypically, which is 3 (5%), 6 (10%) & 2 (3.3%) for *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively (table 3).

Organism	Frequency (%)
*E. col*i	3 (27.3%)
Klebsiella pneumoniae	6 (54.5%)
Pseudomonas aeruginosa	2 (18.2%)
Total	11 (100%)

Table 3: Frequency of *ESBL* among clinical isolates.

Out of 31 imipenem resistant strains 20 (64.5%) clinical isolates showed resistance by modified Hodge test (table 4).

Organism	No of isolates
E. coli	7 (35%)
Klebsiella spp.	8 (40%)
Pseudomonas aeruginosa	5 (25%)
Total	20 (100%)

Table 4: Bacteria showing positive *Carbapenemase* production (No= 31).

Discussion

Resistance to antibiotic drug therapy is an increasing public health problem in all populations. In the recent years, through the abuse and misuse of antibiotics, many bacteria have developed resistance to the variety of antibiotics. This pattern of resistance can be different in various populations and therefore, each of them needs to special program for reduction of resistance to antibiotics especially those are most commonly used for treatment [11]. In the present study, we considered and measured the resistance of some gram-negative bacteria to Imipenem and other selected antibiotics which showed that higher resistance rate against imipenem (17.2%) which is slightly higher than reported with Nair et al., 2013, who found that 12.6% resistance to imipenem. in our study the resistance to Ciprofloxacin, Ceftriaxone and Co-trimoxazole was 59 (32.7%), 77 (42.7%) and 73 (40.5%) which is lower with approximately 50% of the findings of Yadav *et al*., 2015 [12,13].

The frequency of multi-durg resistant among *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 33 (55%), 24 (40%) and 17 (28.3%), respectively. Our percentages are lower than the finding of Yadav et al., 2015 who found that 64(95.52%) and 24(100%) for both E. coli and Klebsiella spp., concurrently, (13). Of particular interest there were considerable percentages of ESBL which detected phenotypically, which was 11 distributed on three clinical isolates as following: (5%), 6 (10%) & 2 (3.3%) for *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. Yadav et al., 2015 reported higher ESBL among E. coli which was 18/67 [13].

Out of 31 isolates, 20 (64.5%) were positive for carbapenemase production by modified Hodge test. Out of MHT positive organisms, the frequency of *Klebsiella* spp. was 40%, followed by *E. coli* (35%) and *Pesudomonas aeruginosa* (25%). The same study was conducted by Amgad et al., 2011who found that 138 out of 200 clinical isolates were MHT positive, the frequency of *E. coli* and *Pseudomonas aeruginosa* was agreed of our results; 38% and 30%, respectively [14]. Whereas the lower carbapenmase production was observed in *Klebsiella* spp. (17%).

Conclusion

Higher carbapenem-resistance was reported 31(17.2%), mostly by Carbapenemase production 20 (64.5%, indicating emerged resistant strains to carbapemens in clinical setting. In addition to

considerable percentages of multi-drug resistant strains and ESBL were also reported.

Recommendations

The prescription of antibiotics should base on susceptibility testing. Complete dose of antibiotic must be taken to prevent resistance. Polices should be taken by health authorities to minimize the antibiotic resistance. Genotyping for MHT negative results is highly recommended to determine the resistance mechanism. Beta-lactamase production test should be done to confirm the phenotypic results of ESBL and followed by genotypic analysis.

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