

Detection of Carbapenem Resistant Gram-Negative Bacilli from Infected Wounds in Khartoum State-2014

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Abstract

Background: Carbapenem family are from the recently synthesized beta-lactam antibiotics which used as last resort antibiotics for treating infections caused by multidrug-resistant Gram-negative bacilli and the resistant to them by Gram-negative bacilli have been developed, due to production of variety of carbapenemase enzymes and other mechanisms that significantly limits treatment options for life-threatening infections.

Objective: This study aims to detect carbapenem resistant Gram-negative rods from infected wounds in Khartoum state and the production of carbapenemase enzymes by the resistant isolates using phenotypic methods.

Method: 100 wound swabs were collected. All samples were cultured directly on blood and MacConkey agar, Cultures were examined macroscopically and microscopically, different standard biochemical tests were performed for identification of Gram-negative bacilli. Standard antimicrobial susceptibility testing to Meropenem antibiotic was done for all Gram-negative bacilli isolates, and Modified Hodge test was performed for the resistant isolates.

Results: 77 Gram-negative bacilli were isolated from 100 samples, the commonest pathogenic isolates were *Proteus* species (28%) followed by *Klebsiella* species (24%), *Escherichia coli* (20%), *Pseudomonas* species (17%), *Enterobacter* species (10%) and *Acinetobacter* species (1%). 13% of the isolates were Carbapenem resistant, and 50% of the resistant isolates were positive for carbapenemase enzymes production using Modified Hodge Test.

Conclusion: The percentage of Carbapenem resistance is high. *Pseudomonas* species followed by *Escherichia coli* were the most carbapenemase producers. Modified Hodge test is simple method for detection of carbapenemase enzymes that can detect many types of carbapenemase but not all types and it does not specify the types. Further studies should be performed using larger sample size and other specific methods especially PCR.

Keywords: Multidrug-resistant; Carbapenem; Carbapenem resistance; Gram-negative bacilli; Carbapenemase enzymes; Modified Hodge test

Introduction

Wound infection is defined by the US Centre for Disease Control and Prevention (CDC) as Surgical Site Infection (SSI) which can be caused by many Gram-positive and Gram-negative bacteria [1].

Clinically important Gram-negative bacilli that can cause wound infections include *Pseudomonas*, *Escherichia*, *Klebsiella*, *Acinetobacter*, *Enterobacter*, *Proteus*, *Providencia* and *Morganella* [2,3].

There are many complications that can occur after wound infection which includes death of surrounding tissue, and septicemia. Septicemia following wound infection may lead to septic shock, a critical illness involving the whole body, which may require intensive care and life support and can lead to multiple organ failure or death [4].

Many antibiotics can be used in the treatment of wound infections that include beta-lactam and non beta-lactam drugs. Beta-lactams include cephalexin, and carbapenem (that are used for the treatment of complicated wound infections). Non beta-lactams include deoxycyclin and clindamycin [5].

Carbapenem is a family of beta-lactam antibiotics generally targets cells by inhibiting transpeptidases (penicillin-binding proteins). This prevents synthesis of peptidoglycan, which is a necessary structural component, leading to cell lysis; this family includes: Imipenem, Meropenem, Ertapenem, and Doripenem [6].

Carbapenem family are from the recently synthesized beta lactam antibiotics used as last resort antibiotics for treating infections caused by multidrug resistant Gram-negative bacilli and the resistance to them have been developed, that significantly limits treatment options for life-threatening infections [7].

Very few studies done in our country regarding carbapenem resistance, and thus why this study is performed.

The resistance mechanisms can be due to production of carbapenemase enzymes, active transport of carbapenem drugs out of

the cell, mutation in or loss of outer membrane porins, preventing antibiotics from entering the cells, and other mechanisms [7].

Carbapenemase enzymes can be detected using different phenotypic methods which include: Modified Hodge Test (MHT), Mast discs ID inhibitor combination disks (MDI), Rosco Diagnostica Neo-Sensitabs KPC and MBL confirmation kit (RDS), Metallo-Beta-Lactamase (MBL) E test, and Ethylene Di amine Tetra acetic acid (EDTA) disc synergy test (EDS), and genotypic methods like Polymerized Chain Reaction (PCR) [8,9].

This study aims to detect Gram negative bacilli causing wound infection, to detect the antimicrobial susceptibility pattern for all Gram-negative bacilli isolates to Carbapenem antibiotics, and to detect carbapenemase enzymes production for the resistant isolates using phenotypic method (Modified Hodge Test).

Materials and Methods

Hospital based cross-sectional study design was conducted in the period from February to April 2014. Ethical clearance has been taken from SUMASRI Institutional Review Board (SIRB) that insures all ethical consideration for conducting the research in a way that protects patient's confidentiality. Hundred wound swabs were collected from patients attended to different hospitals in Khartoum state. Amies transport media was used to ensure the viability of the microorganism from specimen that not examined immediately. All samples were cultured directly in blood agar and MacConkey agar media, and incubated at 37°C over night. Cultures were examined microscopically for colonial morphology, and Gram's stain was performed from suspected colonies, and there is no further identification for bacteria that are not Gram-negative bacilli. Different standard biochemical reactions including oxidase, Kligler Iron Agar (KIA), citrate utilization, urease production, indole production, and motility tests were performed for identification of Gram-negative bacilli. Standard antimicrobial susceptibility testing to Meropenem antibiotic was performed for all Gram-negative isolates using disc diffusion method and Modified Hodge test was performed for the resistant isolates (all media and biochemical tests mentioned above were obtained from Topley House, 52 Wash Lane, Bury, Lancashire, B19 6AU, UK, Meropenem disc was obtained from HI media, UK) [2,3].

Oxidase test

The colony was touched by using sterile wooden stick and it was rubbed in filter paper impregnated with 1% tetramethyl-p-phenylenediamine dihydrochloride reagent. Purple color was developed in 10-60 s was reported positive reaction [2].

Kligler iron agar test (KIA)

A heavy inoculum of the tested colony was streaked over the surface of the slope and stabbed into the butt. The tube was incubated aerobically at 37°C overnight, then the result was interpreted [2].

Citrate utilization tests

Saline suspension of the tested organism was inoculated in slope of simmons citrate agar. The tube was incubated aerobically at 37°C overnight, and then the result was interpreted [2].

Urease production test

Heavy inoculum was inoculated over the entire slope surface of media contain urea. The tube was incubated aerobically at 37°C and examined after 4 h or overnight then the result was interpreted [2].

Indol production test

A heavy inoculum of the tested organism was inoculated in peptone water contain tryptophan, and incubated aerobically at 37°C overnight. 0.5 ml of Kovac's reagent was added to the tube and shaken gently. Then the result was interpreted [2].

Motility test using semi solid media

By using sterile straight wire the tested organism was inoculated making a single stab down the center of the tube to about half the depth of the media, the tube was incubated at 37°C overnight. Then the results were interpreted [2].

Carbapenem susceptibility pattern

The antimicrobial susceptibility to Carbapenem antibiotic was performed using the disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Bacterial colonies was suspended in sterile normal saline in compare to McFarland standard (Oxoid, Eglan) and cultured in Muller-Hinton agar media (obtained from Topley House, UK) using sterile cotton swab, A 10 µg Meropenem disk was put in the center of the media, after overnight incubation at 37°C zone of inhibition was measured and the reading was compared to the sheet provided by manufacturer. Gram negative isolates showed resistant zones to Meropenem were tested for carbapenemase enzyme production by using MHT [2].

Modified hodge test

MHT was used to detect carbapenemase enzyme production.

Procedure

McFarland 0.5 dilution of *E. coli* ATCC 25922 in 5 ml of sterile normal saline was prepared. The suspension was diluted 1:10 and streaked in Muller and Hinton agar media using sterile cotton swab. A 10 µg Meropenem disk was placed in the center of the agar media, then the tested organism was streaked as straight line from the edge of the plate to the disc, the plate was incubated overnight at 37°C.

Positive MHT: Shown by clover leaf-like indentation of *E. coli* susceptible strains growing along the tested organism growth streak within the disk diffusion zone. Negative MHT: no growth of *E. coli* 25922 along the tested organism growth streak within the disc diffusion [10].

Results

Hundred wound swabs were collected from patients attended to hospitals of Khartoum state complaining wound infections. 77 (63%) Gram negative bacilli were isolated Figures 1-3.

Gram negative bacilli isolates

A total of 77 (63%) Gram-negative bacilli isolates comprising of 22 (28%) *Proteus* species 21 (27%) *Proteus mirabilis* and 1 (1%) *Proteus vulgaris*, 18 (24%) *Klebsiella* species 9 (12%) *Klebsiella pneumoniae*, 5

(7%) *Klebsiella oxytoca* and 4 (5%) other *Klebsiella* species, 15 (20%) *E. coli*, 13 (17%) *Pseudomonas* species, 8 (10%) *Enterobacter* species, and 1 (1%) *Acinetobacter* species (Table 1).

Isolated bacteria	Frequency	Percent
<i>Proteus mirabilis</i>	21	27%
<i>E. coli</i>	15	20%
<i>Pseudomonas</i> species	13	17%
<i>Klebsiella pneumonia</i>	9	12%
<i>Enterobacter</i> species	8	10%
<i>Klebsiella oxytoca</i>	5	7%
Other <i>Klebsiella</i> species	4	5%
<i>Proteus vulgaris</i>	1	1%
<i>Acinetobacter</i> species	1	1%
Total	77	100

Table 1: Frequency and percentage of isolated Gram-negative bacilli.

Carbapenem susceptibility pattern

Meropenem susceptibility testing showed that 67 (87%) of Gram-negative rods isolates were Meropenem sensitive and 10 (13%) were Meropenem resistant (Table 2).

Regarding Carbapenem (Meropenem) resistance the most resistant organism was *Pseudomonas* species followed by *E. coli*, *Klebsiella* species and *Acinetobacter* species (Table 2).

Cross tabulation	Susceptibility to Carbapenem antibiotic		Total
	Sensitive	Resistant	
<i>E. coli</i>	12-15.60%	3-3.90%	15-19.50%
<i>Pseudomonas</i> species	9-11.70%	4-5.20%	13-16.90%
<i>Klebsiella</i> species	4-5.20%	0	4-5.20%
<i>Klebsiella pneumoniae</i>	8-10.40%	1-1.30%	9-11.70%
<i>Klebsiella oxytoca</i>	4-5.20%	1-1.30%	5-6.50%
<i>Proteus mirabilis</i>	21-27.30%	0	21-27.30%
<i>Proteus vulgaris</i>	1-1.30%	0	1-1.30%
<i>Enterobacter</i> species	8-10.40%	0	8-10.40%
<i>Acinetobacter</i> species	0	1-1.30%	1-1.30%
Total	67-87%	10-13%	77-100%

Table 2: Cross tabulation between isolated Gram-negative bacilli and susceptibility to Carbapenem.

Carbapenemase enzymes detection (Modified Hodge Test)

MHT was performed for the resistant isolates, 50% of them were positive for enzyme production.

Regarding MHT *Pseudomonas* species were the most carbapenemase producer among the resistant isolates followed by *E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter* species (Table 3).

Cross tabulation	Modified Hodge test		Total
	Positive	Negative	
<i>E. coli</i>	1-10%	2-20%	3-30%
<i>Pseudomonas</i> species	2-20%	2-20%	4-40%
<i>Klebsiella pneumoniae</i>	1-10%	0	1-10%
<i>Klebsiella oxytoca</i>	0	1-10%	1-10%
<i>Acinetobacter</i> species	1-10%	0	1-10%
Total	5-50%	5-50%	10-100%

Table 3: Cross tabulation between isolated Carbapenem resistant Gram-negative bacilli and MHT.



Figure 1: Primary plating showing mixed growth.

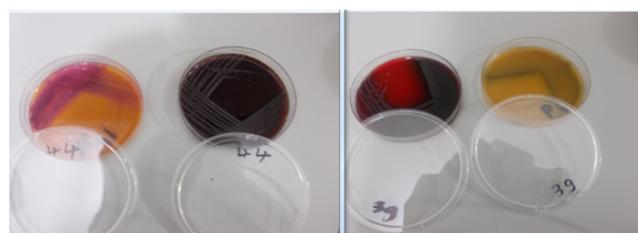


Figure 2: *Pseudomonas* species on blood and MacConkey agar plates showing different pigments.



Figure 3: MHT for Carbapenem resistant isolates showing positive and negative results (example: positive in no.121, negative in no.15).

Discussion

In this study *Proteus mirabilis* was the predominant pathogen among the isolated bacteria. That was not in agreement with other previous studies, in which *Pseudomonas* species and *E. coli* were the commonest isolates from wounds [11,12].

The resistant to Meropenem antibiotics in this study was 13%. This was disagreed with the study conducted by Reham Mohammed, 2013 in Khartoum state; she found that 25.4% of clinical isolates were resistant to Meropenem antibiotic [11], the study conducted by Khanda Abdallatif Anwar in Iraq, 2011 who reported that 22% of the isolates were Meropenem resistant [13]. And also disagreed with the study conducted by Noyal M et al. which found that 43% of the isolates were Meropenem resistant [14].

The most resistant Gram negative bacilli isolates in this study was *Pseudomonas* species (20%). This is in agreement with Reham findings [11]. Other study done by Sharif A et al. in Nigeria reported that *E. coli* was the most resistant organism [12], and the study conducted by Noyal M et al. reported that *Acinetobacter* was the most resistant organism [14].

Regarding MHT 50% of the total Carbapenem resistant isolates were positive that was in agreement with the findings of Gomty Muhajan et al. in India (47.6%) [15].

Many other studies reported lower percentage of positive MHT ranging from 26% to 33.5% [11,12,14], while Amjad A et al. found that 69% of the resistant strains positive for MHT.

Conclusion

This study concludes that the percentage of resistance to Carbapenem antibiotics was high (13%) and cannot be neglected. The most Carbapenem resistant organism and the most carbapenemase producer were *Pseudomonas* species followed by *E. coli*. MHT can detect many types of carbapenemase enzymes effectively but it cannot detect some types and does not specify the enzymes type.

Recommendation

Further studies including other types of clinical specimen, and larger sample size should be tested to cover wider range of isolates.

Other specific tests for detection of carbapenemase enzymes should be used such as EDTA disc synergy test, MDI, RDS, MBL E test, and PCR.

Infectious control program should be performed to prevent the spread of carbapenemase producers.

The use of antibiotics especially broad spectrum antibiotic should be controlled to decrease the emergence of antibiotic resistance and to decrease the emergence of new resistance mechanisms.

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