Carbapenem Resistance Among Gram Negative Bacilli In Lagos; Implications For Antimicrobial Stewardship

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ABSTRACT

Background: The emergence of carbapenem-resistant Gram-negative bacilli (GNB) represents a serious public health threat which requires implementation of antimicrobial stewardship programs to reverse conditions that favour the emergence of multidrug-resistant GNB within the hospital (increased use of carbapenems), thereby reducing morbidity/mortality and healthcare costs. The prevalence of Carbapenem resistant GNB causing infections at LUTH and their resistance pattern to other classes of antimicrobial agents were determined.

Methods: The bacterial isolates were recovered from various clinical specimens in LUTH between January and October 2015. Antimicrobial susceptibility testing was done using the Modified Kirby-Bauer disc diffusion and gradient diffusion methods and interpreted using EUCAST 2014 breakpoints tables, version 4.0 and CLSI 2013 guidelines Carbapenem resistance was defined as resistance to any of imipenem (10µg), meropenem (10 µg) or ertapenem (10 µg).

Result: Four hundred and two Gram- negative bacilli were isolated. Seventy one (17.7%) were carbapenem resistant, comprising 16 (59.3%) of the 27 *Acinetobacter baumanii*, 26 (17%) of the 153 *Pseudomonas aeruginosa*, and 29 (17%) of the 222 *Enterobacteriaece*. All carbapenem resistant isolates were multidrug-resistant except one. Most isolates were susceptible to colistin (88 – 100%), polymixin B (88.5% for *Pseudomonas aeruginosa*), and tigecycline (44.1% for *Enterobacteriaece*).

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Conclusions: There was a high rate of carbapenem resistance among GNB most of which were multi drug resistant. Antimicrobial stewardship should be instituted with the restricted use of carbapenems. Spread of these multi drug resistant organisms should be prevented with infection control practices like hand hygiene and contact based precaution.

INTRODUCTION

Antimicrobial stewardship programmes (ASPs) aim to improve clinical efficacy of antimicrobial treatments and limit antimicrobial resistance through reducing selective pressure which leads to development of resistance, to currently effective antibiotics. Indiscriminate, inadequate and prolonged use of antimicrobials (AMs) leads to emergence and proliferation of resistant strains. Development of antimicrobial resistance pattern is directly proportional to the volume of AM consumed. Therefore, to reduce the development of antimicrobial resistance, usage regulation is essential (1).

Carbapenems are a class of beta-lactam antibiotics with a broad spectrum of antibacterial activity. They are recommended for treatment of severe infections caused by extended-spectrum beta-lactamases (ESBLs) producing Gram- negative bacilli (GNB) (2,3). Carbapenems also became crucial for preventing and treating life-threatening nosocomial infections, which are often associated with techniques developed in modern medicine (transplantation, hospitalization in an intensive care unit, highly technical surgery). Increased prevalence of ESBL producing GNB has led to increased use of carbapenem and the attendant resistance which arises through various mechanisms. These range from overexpression of β-lactamases with no carbapenemase activity to production of carbapenemases with the ability to hydrolyse the carbapenems (4,5); decrease in bacterial outer-membrane permeability – (615) and by active expulsion of antibiotics out of the bacterial cell via increased expression of efflux systems –(14,1624).

The spread of community-acquired Gram negative bacilli (GNB) producing ESBLs capable of hydrolysing almost all β-lactam antibiotics except carbapenems has been reported worldwide (25). The consequence

of this emerging phenomenon has been an increased use of carbapenems (25). These has led to the emergence of carbapenem-resistant Gram-negative bacilli – (2629). Reports of carbapenem resistance worldwide imply that treatment of severe infections especially in association with modern techniques may be jeopardized. (30). Treatment options for patients infected with carbapenem-resistant organisms are very limited and combination therapies comprising two or more classes of antibiotics are often used. –(3134).

Based on this background, the objectives of this study were to determine the prevalence of carbapenem resistant Gram negative bacilli and their antimicrobial susceptibility pattern and to establish the occurrence of multi-drug resistance among carbapenem non-susceptible Gram negative bacilli in a tertiary health care centre in South Western Nigeria.

MATERIALS AND METHODS

STUDY POPULATION

Four hundred and two bacterial isolates belonging to the family *Enterobacteriaceae*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii* were isolated from 377 patients whose clinical specimens were submitted to the Department of Medical Microbiology, Lagos University Teaching Hospital. The bacteria isolates were collected between January and October 2015 and identified using Microbact 24E (Oxoid England). Ethical clearance was obtained from the ethics and research committee of the Lagos University Teaching Hospital. Three hundred and eighty-two specimens were cultured to obtain the bacterial isolates.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility test was performed on the isolates using the Modified Kirby-Bauer disc diffusion methods according to the Clinical and Laboratory Standard Institute (CLSI) recommendation. The test was performed using the commercially available Oxoid® single disc comprising of the following antibiotics: Amikacin (30µg), Amoxillin-clavulanate (20/10µg), Aztreonam (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Ciprofloxacin(5µg), Cefuroxime (30µg), Cefepime (30µg), Cefotaxime (30g), Cefoxitin(30g), Ertapenem (10µg), Gentamicin (10g), Imipenem (10µg), Meropenem (10µg), Nitrofurantoin (300µg), Piperacillin-Tazobactam (100/10µg). Minimal inhibitory concentration (MIC) strips (Liofilchem, Roseto degli Abruzzi, Italy), containing Tigecycline, and Colistin were used to determine the MIC according to the manufacturer's instructions.

The isolates that were nonsusceptible (intermediate or resistant) to any one of the carbapenems, were further tested for susceptibility to the following antibiotics: Enterobacteriaece: Aztreonam (30µg), Tobramycin (10µg), Levofloxacin (5µg), Tigecycline(Etest), and Colistin (Etest) were tested. *Pseudomonas aeruginosa*: Aztreonam (30µg), Tobramycin (10µg), Levofloxacin (5µg), Colistin (10µg, Etest), Polymyxin B (300units) were tested. Acinetobacter spp.: Tobramycin (10µg), Levofloxacin (5µg), Colistin (10µg, Etest), Polymyxin B (300units) were tested. Results were interpreted using EUCAST 2014 breakpoints tables, version 4.0 (36) and CLSI 2013 guidelines [for Colistin (10µg), Polymyxin B (300units)] (37). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as the control strains in susceptibility testing. Multidrug resistance was defined as resistance to at least three classes of antibiotics.

RESULTS

A total of 402 Gram negative bacilli (GNB) comprising 222 (55.2%) isolates of Enterobacteriaceae, 153 (38.1%) *Pseudomonas aeruginosa*, and 27 (6.7%) *Acinetobacter baumannii* were studied. The Enterobacteriaceae comprised of 87(21.6%) *Escherichia coli*, 62 (15.4%) *Klebsiella pneumoniae*, 31 (7.7%) *Klebsiella oxytoca*, 18 (4.5%) *Proteus mirabilis*, 12 (2.9%) *Enterobacter* species, five (1.2%) *Pantoea agglomerans*, two (0.5%) *Proteus vulgaris*, two (0.5%) *Serratia rubidaea*, one (0.3%) *Morganella morganii*, one (0.3%) *Providencia stuartii*, and one (0.3%) *Salmonella enterica ss. arizonae*.

The prevalence of carbapenem resistance in Gram negative bacilli was 71 (17.7%) comprising 16 (59.3%) of the 27 *Acinetobacter baumanii*, 26 (17%) of the 153 *Pseudomonas aeruginosa*, and 29 (13.1%) of the 222 Enterobacteriaceae [16.7% (2/12) *Enterobacter aerogenes*, 10.3% (9/87) *Escherichia coli*, 12.9% (4/31) *Klebsiella oxytoca*, 18.3% (11/62) *Klebsiella pneumoniae*, 20% (1/5) *Pantoea agglomerans*, 5.6% (1/18) *Proteus mirabilis* and 50% (1/2) *Serratia rubidaea*] (see table 1)

Antimicrobial susceptibility testing revealed that the highest susceptibility was observed with imipenem 86.3% and lowest was observed in ciprofloxacin 37.3% for antibiotics tested against all isolates. Among carbapenems, the activity of imipenem (86.3% susceptible) was similar to meropenem (85.6% susceptible), and ertapenem (86.9%) susceptibility amongst *Enterobacteriaece*. As for aminoglycosides, 46% were susceptible to gentamicin compared to 67.4% that were susceptible to amikacin. (Table 2)

All carbapenem resistant isolates were multidrug-resistant (MDR) except one. Multidrug-resistance was mostly to β-lactams (ceftazidime, cefepime, ceftriaxone, cefotaxime), aminoglycosides (amikacin, gentamicin) and fluoroquinolones (ciprofloxacin). They were mostly susceptible to colistin, polymixin B, amikacin and tigecycline (*Enterobacteriaece* only) (Table 3). While less than 50% of *Enterobacteriaece* were sensitive to tigecycline, up to 90% of all isolates were sensitive to colistin.

Table 1: Organisms tested and proportion of which were cabarpenem-resistant

Organisms	Number tested	Cabarpenem-Resistant	Resistance (%)
Escherichia coli	87	9	10.3
Klebsiella pneumoniae	62	11	17.7
Enterobacter aerogenes	12	2	16.7
Klebsiella oxytoca	31	4	12.9
Pantoea agglomerans	5	1	20.0
Proteus mirabilis	18	1	5.6
Serratia rubidaea	2	1	50.0
Morganella morganii	1	0	0
Providencia stuartii	1	0	0
Proteus vulgaris	2	0	0
Salmonella enterica ss. arizonae	1	0	0
Total	222	29	13.1

Table 2: Antimicrobial susceptibility profile of all Gram negative bacilli studied

Antibiotic name	Number tested	S (%)	I (%)	R (%)
Amikacin	402	271 (67.4)	48 (11.9)	83 (20.6)
Amoxicillin/Clavulanic acid	222	35 (15.8)	0	187 (84.2)
Cefepime	402	180 (44.8)	8 (2)	214 (53.2)
Cefotaxime	222	70 (31.5)	4 (1.8)	148 (66.7)
Cefoxitin	222	150 (32.4)	0	72 (67.6)
Cefuroxime	11	8 (72.7)	0	3 (27.3)
Ceftriaxone	222	76 (34.2)	4 (1.8)	142 (64)
Ceftazidime	402	174 (43.3)	26 (6.5)	202 (50.2)
Ciprofloxacin	402	150 (37.3)	12 (3)	240 (59.7)
Ertapenem	222	193 (86.9)	5 (2.3)	24 (10.8)
Gentamicin	402	185 (46)	6 (1.5)	211 (52.5)
Imipenem	402	347 (86.3)	16 (4)	39 (9.7)
Meropenem	402	344 (85.6)	18 (4.5)	40 (10)
Nitrofurantoin	96	60 (62.5)	0 (0)	36 (37.5)
Piperacillin/Tazobactam	402	258 (64.2)	58 (14.4)	86 (21.4)

Table 3: Antimicrobial susceptibility of carbapenem resistant Gram negative bacilli

Antibiotic name	*Enterobacteriacea* % S N= 29	*P. aeruginosa* %S n= (26)	*A. baumannii* %S N=16
Amikacin	62.1	42.3	12.5
Amoxicillin/Clavulanic acid	0	-	-
Ceftriaxone	0	-	-
Ceftazidime	0	23.1	0
Ciprofloxacin	20.7	15.4	0
Cefepime	6.9	15.4	0
Cefotaxime	0	-	-
Cefoxitin	20.7	-	-
Ertapenem	0	-	-
Gentamicin	13.8	19.2	0
Imipenem	31	19.2	12.5
Meropenem	41.4	3.8	0
Nitrofurantoin	13.3	-	-
Piperacillin/Tazobactam	13.8	34.6	0
Aztreonam	6.9	0	-
Levofloxacin	20.7	26.9	6.2
Tobramycin	17.2	15.4	12.5
Polymixin B	-	88.5	-
Colistin	93.1	88.5	100
Tigecycline	41.4	-	-

DISCUSSION

This study shows a high rate of carbapenem resistance in Gram negative bacilli (CRGNB) of 17.7%; this is an increased rate compared with previous studies in the same centre 4.8% in 2010 by Osundiya *et al* (38), 5.2% in 2012 by Oshun *et al (39)* and 15.2% in 2013 by Oduyebo *et al (40)*. These include carbapenem resistant Enterobacteriaceae (CRE), Carbapenem resistant *Acinetobacter baumanii* (CRAB) and carbapenem *Pseudomonas aeruginosa* (CRPsA). The increasing rate of carbapenem resistance calls for caution and drastic preventive actions as carbapenems are among the last line drugs in the treatment of infections by GNB. The use of carbapenems should be protected to reduce the increasing rate of resistance through antimicrobial restriction (either through formulary limitation or by the requirement of pre-authorization and justification) before dispensing. In order to limit the spread of CRGNBs, there should be implementation of hand hygiene and transmission based precautions in management of patients with infections caused by carbapenem resistant GNB (35,41). Due to the high rate of CRGNBs, it will be imperative to implement active surveillance cultures for CRGNBs for patients on admission especially the ICU where the highest rate of resistance was found in this study.

The highest rate of carbapenem resistance was found in *Acinetobacter baumanni* which was about 60%. This is similar to reports from other parts of the world (42). Carbapenem resistant *A. baumanii* is a significant cause of healthcare associated infections in large referral hospitals and poses a major threat to public health (43). One of the key factors that may have led to this high rate of resistance is inappropriate and excessive prescription of antibiotics because most hospitals in Nigeria do not have antibiotic stewardship program in place (44). Moreover, poor infection control practices may contribute to the spread of carbapenem resistance in the hospital environment.

Most of the carbapenem resistant Gram negative bacilli in this study were multidrug resistant. This makes them difficult to treat and this may be associated with increased morbidity and mortality. Furthermore they have the tendency to spread resistance using plasmids and transposons (45). The widespread multi-drug resistance is facilitated by the presence of carbapenemase producing genes on plasmids which also carry genes conferring extended spectrum beta-lactamases, aminoglycoside resistance and fluoroquinolone resistance (45). From this study, they were most susceptible to colistin, polymixin B, amikacin and tigecycline (for enterobacteriaece). In order to reduce mortality associated with infections by carbapenem resistant GNB, optimal and effective antibiotic therapy using Colistin, amikacin, polymyxin B (*P. aeruginosa*), and tigecycline (*Enterobacteriaece*) are advocated and should be tested for. Colistin is very expensive and not readily available in Nigeria while Amikacin, Polymixin B and Tigecycline are available. Although the use of colistin alone is considered to be effective, combination therapies including two or more classes of antibiotics are recommended as significantly more treatment failures were seen in cases that received monotherapy compared to cases who received combination therapy in several studies –(3134).

From the antimicrobial susceptibility of testing in this study, only one third of the Enterobacteriaceae were sensitive to third generation cephalosporin. This implies that 2 out of 3 patient treated with these antibiotics will have treatment failure. The third generation cephalosporins are the most used first line antibiotics in the hospital for treatment of Gram negative infections. Ciprofloxacin which may be an alternative was sensitive in only 37% for Enterobacteriaece. This may contribute to increased use of carbapenems when there is treatment failure.

CONCLUSIONS:

There was a high rate of carbapenem resistance among Gram negative bacilli and most of the CRGNB were multidrug resistant. They were mostly susceptible to colistin, polymyxin B, amikacin and tigecycline and these will be the antibiotics of choice in the treatment of CRGNB. Antimicrobial stewardship should be instituted with the restricted use of carbapenems. Spread of these multi drug resistant organisms should be prevented with infection control practices like hand hygiene and contact based precaution.

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