Journal of Life &Physical Sciences

Available online@www.actasatech.com

acta SATECH 12 (1):14 – 30 (2020)

High Proportion of slime and metallobetalactamase (MβL) producing multi-drug resistant bacteria from patients at referral hospitals in Uyo: A Three-Year Retrospective Study

***Akinjogunla, O.J[1].; Umo, A. N[2]. and Etukudo, I. U[3].**

[1]*Department of Microbiology, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State.*
[2]*Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, University of Uyo, Uyo, Akwa Ibom State.*
[3]*Department of Microbiology, Abia State University, Uturu, Faculty of Biological Sciences, Abia State.*

*Corresponding author: papajyde2000@yahoo.com

Abstract

Bacteriological examination of 510 clinical samples (mid-stream urine (n=194), stool (n=146) and wound swab (n=170) from patients at referral hospitals between 2016 and 2018 was carried out using standard bacteriological techniques. The antibiotic susceptibility profile, slime and metallo-betalactamase (MβL) production among the isolated bacteria were determined by disc diffusion, Congo Red Agar (CRA) / Tube Method and Imipenem / ethylenediamine-tetra-acetic acid disc methods, respectively. A total of 564 bacteria constituting eight genera were isolated from mid-stream urine (n=194), stool (n=146) and wound (n=170) specimens. *Escherichia coli* (37.3%) was the predominant species while *Acinetobacter* spp (7.1%) and *Citrobacter freundii* (7.1 %) were the least occurring species. The results showed that ≥ 46.8 %, 37.9 % and ≥ 34.0 % isolates were resistant to Imipenem; Ampicillin and Ceftriaxone., respectively. Out of 564 isolates obtained, 59.4% were multidrug resistant strains with multiple antibiotic resistant indices ranging from 0.38 to 0.63. Of 270 Imipenem resistant isolates, 64.4% and 65.9% isolates were positive for MβL production by the double disc synergy test and combined double disc test, respectively. The percentages of slime producing isolates using CRA in decreasing order were *K. pneumoniae* (82.9%) > *Acinetobacter* spp (77.8%) > *E. coli* (64.2%) > *P. aeruginosa* (62.5%) > *P. mirabilis* (51.7 %) > *Salmonella* spp (45.0%) >*Enterobacter* spp (36.8%) > *C. freundii* (33.3%). Quantitative assessment of the 352 isolates revealed 20% as weak, 34% as moderate and 47% as strong slime producers. This study therefore showed the necessity to continuously assess the antibiotic susceptibility profiles of bacterial slime producers and also to consider the use of non-carbapenem antibiotics for treatment of infectious diseases.

Key Words: Slime, Metallobetalactamase, Resistant, Isolates, Antibiotics, Imipenem.

Introduction

Microorganisms make use of different mechanisms for their survival in the environment and in humans (Hall-Stoodley *et al*., 2004). Slime production by bacteria is one of the mechanisms by which microorganisms adhere to and colonize smooth artificial surfaces such as indwelling prosthetic devices and catheters. Slime is a viscous, loosely bound, extracellular material comprising exopolysaccharides, glycoproteins and glycolipids (Akinjogunla *et al*., 2018). Studies have shown that slime-producing bacteria have a higher colonization capacity than the non-slime-producing isolates (Ammendolia *et al*., 1999; Arslan and Özkarde, 2007). Thus, bacterial slime plays a role in the establishment and severity of infections (Ammendolia *et al*., 1999), aids in bacterial resistance to phagocytosis and opsonization and protects bacteria from antibiotics and unfavourable environmental conditions (Akyar *et al*., 1998). Some strains of *Staphylococcus* spp., *Acinetobacter* spp., *Escherichia coli* and *Pseudomonas aeruginosa* have been reported as slime producers (Ammendolia *et al*., 1999; Arslan and Özkarde, 2007). Bacterial slime has been associated with intravenous-catheter-related bacteremia and other prosthetic device infections (Rupp and Archer, 1994).

Antibiotic resistance by bacteria is a serious threat to management of patients' illnesses worldwide. Bacteria have developed various mechanisms of resistance to antibiotics such as production of beta-lactamases, alteration of penicillin-binding proteins and plasmid enzymatic modification (Akinjogunla *et al*., 2011). Metallo-β-lactamases (MβLs) are zinc-dependent enzymes, belonging to Amblers Class B, which hydrolyze a variety of beta-lactam antibiotics such as penicillins, cephalosporins and carbapenems, and require divalent cation (zinc) as a cofactor for enzyme activity (Bush *et al*., 1995; Navon-Venezia *et al.,* 2006). These β-lactamase enzymes are produced by bacteria, especially Gram-negative bacteria, and the clinical infections with MβL-producing isolates are associated with high morbidity and mortality (Deshmukh *et al*., 2011). Carbapenem resistance due to MβL production has been recently reported among clinical isolates of *E. coli*, *P. aeruginosa* and *Klebsiella* spp. (Navon-Venezia *et al.,* 2006; Villegas *et al*., 2007). Therefore, the presence of MβL-producing bacteria in the hospital environment has put the use of the beta-lactam antibiotics especially carbapenems under threat (Thompson, 2010). This study was therefore designed to determine the occurrence of slime and MβL-producing producing MDR bacterial isolates in clinical specimens from 2016 to 2018 in Uyo, Nigeria.

Materials and methods

Collection of specimens

Five hundred and ten (510) clinical specimens consisting of mid-stream urine (n =97), stool (n =73) and wound swab (n =85) were aseptically collected using sterile wide-mouth vials (100mL) and swab sticks from patients at referral hospitals in Uyo, Akwa Ibom State. The Hospital Ethics Review Committee approval and verbal informed consent of patients were obtained prior to samples collection. The clinical specimens were carefully labelled to reflect the date and time of collection and were immediately transported to the Microbiology Laboratory, University of Uyo, for bacteriological analysis.

Bacteriological Analysis of Samples

The methods described by Jena *et al.* (2013) was used for the isolation of urinary bacterial isolates. One millilitre (1 mL) of each well mixed mid-stream urine (MSU) specimens was inoculated onto plates of cysteine lactose electrolyte deficient agar (CLED) and MacConkey agar (MCA) without salt. Each of the wound swabs was immersed into a test tube containing 9 mL of sterile distilled water; 0.1 mL was pipetted and surface-inoculated on plates of MCA and blood agar. One gram of each stool specimen was serially diluted and one millilitre (1 mL) of each aliquot was inoculated on plates of MCA and Eosine Methylene Blue (EMB) agar. All the plates were aerobically incubated for 24hrs at 37ºC. After incubation, plates with positive bacterial growths were subcultured onto plates of nutrient agar and incubated for 24 hrs at 37ºC. Thereafter, pure cultures of isolates were streaked onto nutrient agar slants, incubated for 24 hrs at 37ºC and stored in the refrigerator at 4°C until identification. All isolates were Gram stained and subjected to various conventional biochemical tests using standard methods as described in Cheesbrough (2006).

Antibiotic susceptibility testing

In vitro susceptibility testing of all the bacterial isolates to Ampicillin (AMP, 10 μg), Imipenem (IMP, 10 μg), Meropenem (MER, 10 μg), Ceftriaxone (CTX, 30 μg), Streptomycin (STP, 10 μg), Ceftazidime (CTZ, 30 μg), Ciprofloxacin (CIP, 5 μg) and Gentamycin (GEN, 10 μg) (Oxoid, UK) was determined by the disc diffusion method (CLSI, 2009). Briefly, 10 μL of each bacterial isolate, prepared directly from an 18-hr agar plate and adjusted to 0.5 McFarland Standard, was spread-plated on Mueller Hilton Agar (MHA). The antibiotic discs were aseptically placed on the surfaces of the culture plates with sterile forceps, and the plates were incubated at 37°C for 18 hrs. Thereafter, inhibitory zones were observed and measured in millimeters (mm). The interpretation of the measurement as sensitive and resistant was determined based on the criteria of CLSI (2009).

Determination of multiple antibiotic resistance Index

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=*x/y,* where '*x'* was the number of antibiotics to which test isolate displayed resistance and '*y'* was the total number of antibiotics to which the test isolates has been evaluated for sensitivity (Akinjogunla *et al.,* 2010). Isolates that were resistant to three or more antibiotic classes were taken to be multiple antibiotic resistant (Jan *et al*., 2002).

Phenotypic detection of metallo-betalactamase Using combined double disc test

The metallo-betalactamase (MβL) producing Gram negative bacterial (GNB) isolates was determined using Combined Double Disc Test (CDDT). Ten microlitres (10 µL) of each GNB isolates, prepared directly from an overnight agar plate, adjusted to 0.5 McFarland Standard, was spread-plated on MHA. A disc of imipenem (10 µg) and imipenem-EDTA disc (imipenem disc impregnated with 5 µL of EDTA) were placed 20 mm apart on the plates. After incubation for 18 hr at 37°C, a difference of ≥ 7 mm between inhibitory zones of the imipenem and imipenem-EDTA discs indicated MβL production (Deshmukh *et al*., 2011; Sachdeva *et al.,* 2017).

Phenotypic detection of metallo-betalactamase using double disc synergy test (DDST)

The production of MβL by GNB isolates was determined using the Double Disc Synergy Test (Deshmukh *et al*., 2011; Sachdeva *et al*., 2017). Ten microlitres (10 µg) of each GNB isolate prepared directly from an agar plate incubated for 18 hours and adjusted to 0.5 McFarland Standard was spread-plated on MHA. Imipenem (10 µg) and EDTA discs (a blank filter paper disc impregnated with 10 µL of 0.5M EDTA solution) were placed 20 mm apart on the plates. After incubation for 18 hr at 37°C, an enlarged inhibitory zone towards the EDTA disc was considered MβL positive.

Phenotypic detection of slime using congo red Agar method

Slime production by GNB isolates was evaluated by the Congo red agar (CRA) method previously described by Akinjogunla *et al*. (2018). The medium was prepared with 37 g/L brain heart infusion (BHI) broth, 50 g/L sucrose, 10 g/L agar and 0.8 g/L Congo red. Plates of CRA were surface-inoculated with test isolates and incubated aerobically for 24 hrs at 37 ^{o}C. A positive result was indicated by black colonies with a dry crystalline consistency.

Phenotypic detection of slime using tube method (Quantitative Assessment)

Slime production by GNB isolates was determined using the Tube Method (TCM) of Hassan *et al.*

(2011) with slight modification. Briefly, a loopful of each GNB isolate from 24 hrs agar plate was inoculated onto a test tube containing 10 mL BHI broth supplemented with 2% sucrose. The tubes were incubated aerobically for 24 hrs at 37 ^{o}C. After incubation, the contents of each tube were poured out, each tube washed with phosphate buffer saline (pH 7.3), dried and stained with crystal violet (0.1%). Excess stain in each tube was washed off with deionized water and tubes dried in an inverted position. A positive result for slime formation was indicated when a visible film lined the wall and bottom of the tube; quantification was as follows: 0: absent; 1: weak; 2: moderate and 3: strong.

Statistical analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0. Armonk, NY: IBM Corp.) was used for data analysis. The significant difference between metallo-betalactamase and non-metallo-betalactamase producing bacterial isolates at $p \leq 0.05$ were determined using chi-square ($\chi 2$) test. Descriptive data were presented as percentages.

Results

A total of 564 bacteria, belonging to eight genera, were isolated from MSU (n=194), Stool (n=146) and wound (n=170) specimens within the study period (Table 1). *Escherichia coli* was the predominant bacterial isolate constituting 37.3% (190/564), followed by *P. aeruginosa* and *K. pneumoniae* with 18.8% (96/564) and 13.7% (70/564), respectively. Of the 58 isolates identified as *P. mirabilis*, 30 were isolated from wound swabs, 18 from MSU, while 10 were from stool specimens. *Salmonella* spp, *Enterobacter* spp, *C. freundii* and *Acinetobacter* spp constituted 7.8%, 7.5%, 7.1% and 7.1% of the total bacterial isolates (n=564), respectively (Table 1).

The results of the antibiotic resistant profiles of clinical bacterial isolates obtained between 2016 and 2018 are presented in Table 2. The results showed that > 46.8% isolates were resistant to Imipenem and Meropenem (carbapenem class of beta-lactam); 37.9% isolates were resistant to Ampicillin (penicillin class), > 34.0% isolates showed resistance to Ceftriaxone and Ceftazidime (cephalosporin class); 22.3% isolates were resistant to Ciprofloxacin (fluoroquinolone class), while > 19.1% bacterial isolates were resistant to Gentamycin and Streptomycin (aminoglycoside class). Table 3 shows the MAR indices of bacterial isolates from clinical specimens within the study period. Of the 564 bacterial isolates obtained, 72 (12.8%) were sensitive to all the 8 antibiotics tested, 92 (16.3%) were single drug resistant isolates, 65(11.5%) isolates were resistant to 2 antibiotics, while 335 (59.4%) isolates demonstrated multidrug resistant (MDR) phenotypes. The MAR indices of *Acinetobacter* spp and *Enterobacter* spp ranged

from 0.38 to 0.63 (≤ 5 antibiotics) and 0.38 to 0.75 (≤ 6 antibiotics), respectively. The MAR indices of *Salmonella* spp and *C. freundii* ranged between 0.38 and 0.88, while 14 isolates comprising *E. coli* (n=7), *K. pneumoniae* (n=2), *P. aeruginosa* (n=4) and *P. mirabilis* (n=1) had the highest MAR index of 1.0 (Table
3).

Out the 270 Imipenem resistant bacterial isolates from the clinical specimens, 174 (64.4%) isolates were positive for MβL production by DDST. Of the 174 phenotypically detected MβL producing bacterial isolates, *E coli* had (70), *K. pneumoniae* (16), *P. aeruginosa* (32), *Salmonella* spp (10), *P. mirabilis* (14), *Enterobacter* spp (10), *C. freundii* (10) and *Acinetobacter* spp (12). Out the 270 Imipenem resistant bacterial isolates from the clinical specimens, 178 (65.9%) isolates were positive for MβL production by CDDT. More than 74.0% *E. coli* and *Acinetobacter* spp obtained from the clinical specimens were MβL producers, while < 57.1 % *K. pneumoniae*, *Salmonella* spp and *P. mirabilis* were positive for MβL production by CDDT (Table 4). There was no significant difference ($p > 0.05$) between MβL producers and non- MβL producers using DDST and CDDT (Table 4).

The occurrence of slime producing bacterial isolates in clinical specimens using Congo Red Agar (CRA) is presented in Table 5. The results showed a statistically significant difference ($p < 0.05$) between the slime producers (342, 60.6%) and non-slime producers (222, 39.4 %). The percentage proportion of slime producing bacterial isolates using CRA in decreasing order was *K. pneumoniae* (82.9%) > *Acinetobacter* spp (77.8%) > *E. coli* (64.2%) > *P. aeruginosa* (62.5%) > *P. mirabilis* (51.7 %) > *Salmonella* spp (45.0%) >*Enterobacter* spp (36.8%) > *C. freundii* (33.3%) (Table 5). Three hundred and fifty-two (62.4 %) bacterial isolates from clinical specimens produced extracellular material (slime) using tube method (TCM). The quantitative assessment of the 352 slime producers revealed 70 (19.9%) as weak (+), 118 (33.5%) as moderate (++) and 164 (46.6%) as strong (+++) slime producers. The proportion of slime producing bacterial isolates from clinical specimens was significantly ($p=0.0002$) greater than non-slime producers using TCM (Table 6).

Table 1: Occurrences of bacterial isolates in clinical specimens from patients at referral hospitals

Bacterial Isolates	No (%) of Occurence			Total
	Urine (n =194)	Stool (n =146)	Wound (n =170)	No (%)
Escherichia coli	72 (37.1)	90 (61.6)	28 (16.5)	190 (37.3)
Klebsiella pneumoniae	26 (13.4)	24 (16.4)	20 (11.8)	70 (13.7)
Pseudomonas aeruginosa	38 (19.6)	16 (10.9)	42 (24.7)	96 (18.8)
Salmonella spp	22 (11.3)	18 (12.3)	0 (0.0)	40 (7.8)
Proteus mirabilis	18 (9.3)	10 (6.8)	30 (17.6)	58 (11.4)
Enterobacter spp	12 (6.2)	14 (9.6)	12 (7.1)	38 (7.5)
Citrobacter freundii	8 (4.1)	20 (13.7)	8 (4.7)	36 (7.1)
Acinetobacter spp	18 (9.3)	6 (4.1)	12 (7.1)	36 (7.1)
Total	214	198	152	564 (100)

Table 2: Antibiotic resistance profiles of clinical bacterial isolates from patients at referral hospitals

Bacterial Isolates	Year	No of Isolates	Number (Percentage) of Isolates Resistant to Antibiotics							
			AMP	GEN	IMI	CTX	CTZ	STP	MER	CIP
E. coli	2016	52	20 (38.5)	14 (26.9)	28 (53.8)	18 (34.6)	20 (38.5)	12 (23.1)	26 (50.0)	14 (26.9)
	2017	62	22 (35.5)	10 (16.1)	34 (54.8)	18 (29.0)	26 (41.9)	10 (16.1)	34 (54.8)	18 (29.0)
	2018	76	30 (39.5)	16 (21.1)	38 (50.0)	26 (34.2)	26 (34.2)	14 (18.4)	36 (47.4)	16 (21.1)
K. pneumonia	2016	18	6 (33.3)	4 (22.2)	8 (44.4)	6 (33.3)	4 (22.2)	4 (22.2)	8 (44.4)	2 (11.1)
	2017	22	10 (45.4)	4 (27.3)	10 (45.4)	8 (36.4)	10 (45.4)	2 (9.1)	10 (45.4)	4 (27.3)
	2018	30	12 (40.0)	4 (13.3)	14 (46.7)	12 (40.0)	10 (33.3)	4 (13.3)	16 (53.3)	6 (20.0)
P. aeruginosa	2016	26	12 (46.2)	8 (30.8)	14 (53.8)	14 (53.8)	12 (46.2)	8 (30.8)	14 (53.8)	8 (30.8)
	2017	40	16 (40.0)	6 (15.0)	18 (45.0)	16 (40.0)	16 (40.0)	6 (15.0)	16 (40.0)	6 (15.0)
	2018	30	10 (33.3)	2 (6.7)	12 (40.0)	8 (26.7)	8 (26.7)	6 (20.0)	16 (53.3)	4 (13.3)
Salmonella spp	2016	10	4 (40.0)	2 (20.0)	4 (40.0)	4 (40.0)	2 (20.0)	2 (20.0)	4 (40.0)	2 (20.0)
	2017	10	4 (40.0)	2 (20.0)	4 (40.0)	4 (40.0)	4 (40.0)	4 (40.0)	4 (40.0)	2 (20.0)
	2018	20	6 (30.0)	4 (20.0)	8 (40.0)	8 (40.0)	6 (30.0)	4 (20.0)	10 (50.0)	4 (20.0)
P. mirabilis	2016	14	6 (42.9)	6 (42.9)	8 (57.1)	4 (28.6)	10 (71.4)	2 (14.3)	6 (42.9)	6 (42.9)
	2017	30	10 (33.3)	6 (20.0)	14 (46.7)	10 (33.3)	8 (26.7)	6 (20.0)	12 (40.0)	8 (26.7)
	2018	14	4 (28.6)	2 (14.3)	6 (42.9)	6 (42.9)	8 (57.1)	4 (28.6)	8 (57.1)	2 (14.3)
Enterobacter spp	2016	10	4 (40.0)	2 (20.0)	4 (40.0)	0 (0.0)	4 (40.0)	0 (0.0)	4 (40.0)	2 (20.0)
	2017	16	8 (50.0)	6 (37.5)	8 (50.0)	4 (25.0)	6 (37.5)	2 (12.5)	6 (37.5)	6 (37.5)
	2018	12	4 (33.3)	0 (0.0)	4 (33.3)	4 (33.3)	2 (16.7)	4 (33.3)	4 (33.3)	0 (0.0)
C. freundii	2016	18	6 (33.3)	4 (22.2)	8 (44.4)	4 (22.2)	2 (11.1)	8 (44.4)	6 (33.3)	6 (33.3)
	2017	8	2 (25.0)	0 (0.0)	4 (50.0)	2 (25.0)	4 (50.0)	2 (25.0)	4 (50.0)	2 (25.0)
	2018	10	4 (40.0)	0 (0.0)	4 (40.0)	2 (40.0)	4 (40.0)	0 (0.0)	4 (40.0)	0 (0.0)
Acinetobacter spp	2016	4	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)
	2017	12	4 (33.3)	2 (16.7)	6 (50.0)	6 (50.0)	4 (33.3)	2 (16.7)	4 (33.3)	4 (33.3)
	2018	20	8 (40.0)	4 (20.0)	10 (50.0)	8 (40.0)	8 (40.0)	6 (30.0)	10 (50.0)	4 (20.0)

Keys: AMP: Ampicillin, GEN: Gentamycin; IMI: Imipenem; CTX: Ceftriaxone; CTZ: Ceftazidime; STR: Streptomycin; MER: Meropenem; CIP: Ciprofloxacin.

Table 3: Multiple antibiotic resistant index of bacterial isolates in clinical specimens from patients at referral hospitals

MAR Index	Number / Percentage								Total
	EC	KP	PA	SS	PM	ES	CF	AS	No (%)
0.00	24(12.6)	8(11.4)	11(11.4)	3(7.5)	7(12.1)	5(13.2)	5(13.9)	9(25.0)	72(12.8)
0.13	28(14.7)	10(14.3)	15(15.6)	7(17.5)	10(17.2)	6(15.8)	9(25.0)	7(19.4)	92(16.3)
0.25	19(10.0)	7(10.0)	10(10.4)	7(17.5)	9(15.5)	4(10.5)	4(11.1)	5(13.9)	65(11.5)
0.38	43(22.6)	18(25.7)	24(25.0)	10(25.0)	8(13.8)	7(18.4)	7(19.4)	9(25.0)	126(22.3)
0.50	30(15.8)	10(14.3)	13(13.5)	3(7.5)	10(17.2)	4(10.5)	4(11.1)	3(8.3)	77(13.7)
0.63	11(5.8)	6(8.6)	6(6.3)	2(5.0)	4(6.9)	7(18.4)	2(5.6)	3(8.3)	41(7.3)
0.75	18(9.5)	4(5.7)	9(9.4)	7(17.5)	7(12.1)	5(13.2)	4(11.1)	0(0.0)	54(9.6)
0.88	10(5.3)	5(7.1)	4(4.2)	1(2.5)	2(3.4)	0(0.0)	1(2.8)	0(0.0)	23(4.1)
1.00	7(3.9)	2(2.9)	4(4.2)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	14(2.5)

Keys: EC: *E. coli*; KP: *K. pneumoniae*; PA: *P. aeruginosa;* SS: *Salmonella* spp; PM: *P. mirabilis*; ES: *Enterobacter* spp; CF: *C. freundii*; AS: *Acinetobacter* spp

Table 4: Metallo-betalactamase (MβL)-producing bacteria in clinical specimens from patients at referral hospitals

Method Used	Bacterial Isolates	No of IMP^R Isolates	MβL Producers No (%)	Non-MβL Producers No (%)	χ2	p-value
DDST	*E. coli*	100	70 (70.0)	30 (30.0)	8.25	0.311
	K. pneumoniae	32	16 (50.0)	16 (50.0)		
	P. aeruginosa	44	32 (72.7)	12 (27.3)		
	Salmonella spp	16	10 (62.5)	6 (37.5)		
	P. mirabilis	28	14 (50.0)	14 (50.0)		
	Enterobacter spp	16	10 (62.5)	6 (37.5)		
	C. freundii	16	10 (62.5)	6 (37.5)		
	Acinetobacter spp	18	12 (66.7)	6 (33.3)		
	Total	270	174 (64.4)	96 (35.6)		
CDDT	*E. coli*	100	74 (74.0)	26 (26.0)	10.5	0.162
	K. pneumoniae	32	16 (50.0)	16 (50.0)		
	P. aeruginosa	44	28 (63.6)	16 (36.2)		
	Salmonella spp	16	8 (50.0)	8 (50.0)		
	P. mirabilis	28	16 (57.1)	12 (42.9)		
	Enterobacter spp	16	10 (62.5)	6 (37.5)		
	C. freundii	16	10 (62.5)	6 (37.5)		
	Acinetobacter spp	18	14 (77.8)	4 (22.2)		
	Total	270	178 (65.9)	92 (34.1)		

Keys: DDST: Double Disc Synergy Test: CDDT: Combined Double Disc Test; MβL: Metallobetalactamase; IMP: Imipenem

Table 5: Slime producing bacterial isolates in clinical specimens (Congo Red Agar Method) from patients at referral hospitals

Bacterial Isolates	No of Isolates	Slime Producers No (%)	Non-Slime Producers No (%)	χ2	p-value
E. coli	190	122 (64.2)	68 (35.8)		
K. pneumoniae	70	58 (82.9)	12 (17.1)		
P. aeruginosa	96	60 (62.5)	36 (37.5)		
Salmonella spp	40	18 (45.0)	22 (55.0)		
P. mirabilis	58	30 (51.7)	28 (48.3)	46.4	0.0001*
Enterobacter spp	38	14 (36.8)	24 (63.2)		
C. freundii	36	12 (33.3)	24 (66.7)		
Acinetobacter spp	36	28 (77.8)	8 (22.2)		
Total	564	342 (60.6)	222 (39.4)		

Table 6: Slime producing bacterial isolates in clinical specimens (Tube Method) from patients at referral hospitals

Bacterial Isolates	No of Isolates	Slime Producers	Quantitative Assessment of Slime Producers			Non-Slime Producers	χ2	p-value
			Weak	Moderate	Strong			
		No (%)	No (%)	No (%)	No (%)	No (%)		
E. coli	190	126 (66.3)	26 (20.6)	38 (30.2)	62 (49.2)	64 (33.7)		
K. pneumonia	70	58 (82.9)	16 (27.6)	18 (31.0)	24 (41.4)	12 (17.1)		
P. aeruginosa	96	60 (62.5)	12 (20.0)	26 (43.3)	22 (36.7)	36 (37.5)		
Salmonella spp	40	16 (40.0)	4 (25.0)	4 (25.0)	8 (50.0)	24 (60.0)		
P. mirabilis	58	32 (55.2)	4 (12.5)	14 (43.8)	14 (43.8)	26 (44.8)	38.5	0.0002*
Enterobacter spp	38	18 (47.4)	2 (11.1)	6 (33.3)	10 (55.6)	20 (52.6)		
C. freundii	36	14 (38.9)	0 (0.0)	4 (28.6)	10 (71.4)	22 (61.1)		
Acinetobacter spp	36	28 (77.8)	6 (21.4)	8 (28.6)	14 (50.0)	9 (22.2)		
Total	564	352 (62.4)	70 (19.9)	118 (33.5)	164 (46.6)	212 (37.6)		

Discussion

The clinical and financial burden of patients and health care providers at referral hospitals with slime (pathogenic) and MβL producing MDR bacterial isolates is enormously challenging. In our study, *E. coli* was the predominant bacterial isolate from the clinical samples, followed by *P aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *Salmonella* spp, *Enterobacter* spp, *Acinetobacter* spp and *C. freundii*. *E. coli* as the predominant bacterial uropathogen in this study was consistent with similar studies conducted by Assefa *et al*. (2008) and Akinjogunla and Divine-Anthony (2013). The isolation of *Acinetobacter* spp in stool, wound and urine samples was in conformity with results of Dijkshoorn *et al*. (2005); Howard *et al*. (2012) and Jimenez-Guerra *et al*. (2018) who isolated *Acinetobacter* spp from stool, wound and urine samples in Netherland, Ireland and Spain, respectively.

This study showed a high Imipenem, Meropenem, Ampicillin**,** Ceftriaxone and Ceftazidime resistant isolates. The overall resistance of isolates to Imipenem and Meropenem was ≥ 46.8 % and this value was higher than 17 % and ≥ 22% reported by Manoharan *et al.* (2011) and Gupta *et al.* (2006), respectively. Of the 335 MDR bacterial isolates obtained, *E. coli* had the highest percentage with 35.5%, followed by *P. aeruginosa* (17.9 %) and this substantiated the findings of Aly and Balkhy, (2002) who reported *E. coli* as the Gram negative bacilli (GNB) with the highest MDR profile, but contradicted Qadri *et al.* (1996) who had *P. aeruginosa* exhibiting the highest MDR profile in Riyadh, Saudi Arabia.

The rapid increasing rate of MβL, zinc-dependent enzymes belonging to Amblers Class B, production among bacterial isolates in the family Enterobacteriaceae has become a grave public health problem worldwide and has made use of beta-lactam antibiotics especially carbapenems for treatment of infectious diseases under threat (Bush *et al*., 1995; Thompson, 2010). In our study, MβL producing *K. pneumoniae*, *P. aeruginosa*, *Salmonella* spp, *P. mirabilis*, *Enterobacter* spp, *E coli*, *C. freundii* and *Acinetobacter* spp were obtained from the clinical samples using DDST and CDDT. The phenotypic detection of MβL production in GNB such as *E coli*, *K. pneumoniae* and *Salmonella* spp in this study agreed with the results of Hoang *et al*., (2013). The occurrence of MβL producing C. *freundii* and *Acinetobacter* spp corroborated the findings of Jena *et al.* (2013) who isolated 75 % *C. freundii* and 25 % *Acinetobacter* spp producing MβL from urine samples in India. More than 63.6 % *P. aeruginosa* obtained in this study were positive for MβL production and this value was higher than 41.0 % and

11.1 % reported by Sadhana *et al.* (2014) and Zubair and Iregbu (2018), respectively.

Microorganisms that produce slime and form biofilms have mechanisms to synthesize several molecules that facilitate its survival in humans (Chalabaev *et al*., 2014). In this study, varied percentages of slime producing *K. pneumoniae, Acinetobacter* spp., *E. coli, P. aeruginosa, P. mirabilis, Salmonella* spp, *Enterobacter* spp and *C. freundii* were obtained from clinical samples. The occurrence of slime producing *E. coli* and *K pneumoniae* agreed with Dadawala *et al.* (2010) and Hassan *et al.* (2011) whose findings showed slime producing *E. coli* and *K pneumoniae* in stool and urine samples in India using CRA and TCM.

Conclusion

The study has established a high occurrence of slime- and MβL-producing MDR bacterial isolates in Uyo. Hence, it is of necessity to consider other antibiotics besides carbapenem class of beta-lactam for treatment of infectious diseases in Nigeria. Furthermore, routine check of the antibiotic susceptibility profiles of slime-producing bacteria is necessary to support the choice of antibiotics selected for effective treatment of infectious diseases.

References

Afreenish, H., Javaid U., Kaleem, F., Omair, M., Khalid, A. and Muhammad, I. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates *Brazilian Journal of Infectious Diseases*, 15(4):305-311.

Akinjogunla, O. J., Ehinmore, I., Atang, T.D. and Akpan, I. (2018). Antibacterial efficacy of aqueous leaf extracts of *C. citratus* (Poaceae) and *B. verticillata* (Rubiaceae) on biofilm producingg uropathogenic bacterial isolates. *World Journal of Applied Science and Technology*. 10(1b):1-8.

Akinjogunla, O. J., Odeyemi, A.T. and Olasehinde, G. I. (2010). Epidemiological studies of Urinary tract infections among post-menopausal women in Uyo, South- South, Nigeria, *Journal of American Science,* 6 (12):1674-1681.

Akinjogunla, O.J., Eghafona, N.O. and Enabulele, I.O. (2011). Aetiologic agents of acute otitis media (AOM): prevalence, antibiotic susceptibility, β-lactamase (βl) and extended spectrum β-lactamase (ESβL) production. *The Journal of Microbiology, Biotechnology and Food Sciences*, 1 (3) 333-353.

Akinjogunla, O.J. and Divine-Anthony, O. (2013). Asymptomatic bacteriuria among apparently healthy undergraduate students in Uyo, South-South, Nigeria. *Annual Review and Research in Biology*, 3(3): 213-225.

Akyar, I., Fidan, I. and Rota, S. (1998). Koagülaznegatif stafilokoklarda slime faktör yapiminin üç farkli yöntemle aras¸tirilmasi, tür tayini ve antibiyotik direnci. *Mikrobiyol Bült*.; 32:15– 22.

Aly, M. and Balkhy, H.H. (2002). The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. *Antimicrobial Resistance and Infection Control*, 1:26.

Ammendolia, M.G., Dirosa, R., Montanaro, R., Arciola, C.R. and Baldassarri, L (1999). Slime production and expression of the slime-associated antigen by Staphylococcal clinical isolates. *Journal of Clinical Microbiol*ogy, 37: 3235-3238.

Arslan, S. and Özkarde, F. (2007). Slime production and antibiotic susceptibility in Staphylococci isolated from clinical samples, *Mem Inst Oswaldo Cruz, Rio de Janeiro*, 102(1): 29-33.

Assefa, A., Asrat, D., Woldeamanuel, Y. G., Hiwot, Y., Abdella, A. and Melesse, T. (2008). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialized Hospital Addis Ababa, *Ethiopian Journal of Medical*, 46(3):227-235.

Bush, K., Jacoby, G. A. and Medeiros, A. A. (1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*, 39:1211-1233.

Chalabaev, S., Chauhan, A. and Novikov, A. (2014). Biofilms formed by Gram-negative bacteria undergo increased lipid A palmitoylation, enhancing in vivo survival. *mBio*., 5:1-14.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries* (Part II). Cambridge University pp. 19 - 110.

Clinical Laboratory Standards Institute (2009). Method for antifungal disk diffusion Susceptibility testing of Yeasts; Approved Guideline-(2nd Ed). CLSI document M44-A2. Clinical Laboratory Standard Institute, Wayne: Pennsylvania.

Dadawala, A.I., Chauhan H.C., Chandel, B.S., Ranaware, P., Patel, S.S, Khushboo, S., Rathod, P.H., Shah N.M. and Kher, H.N. (2010). Assessment of *Escherichia coli* isolates for In vitro biofilm production, *Veterinary World*, 3(8): 364-366.

Deshmukh, D.G., Damle, A.S., Bajaj, I.K., Bhakre, J.B. and Patwardhan, N.S. (2011). Metallo-β-lactamase-producing clinical isolates from patients of a tertiary care hospital. *Journal of Laboratory Physicians*, 3 (2): 93-97.

Dijkshoorn, L., Aken, E. V., Shunburne, T.J., der-Reijden, K., Bernards, A.T., Nemec, A. and Towner, K.J. (2015). Prevalence of *Acinetobacter baumannii* and other *Acinetobacter* spp. in faecal samples from non-hospitalized individuals. *Control Microbiology and Infection*, 11 (4):329-332.

Gupta, E., Mohanty, S., Sood, S., Dhawan, B., Das, B.K. and Kapil, A. (2006). Emerging resistance to carbapenems in a tertiary care hospital in north India. *Indian Journal of Medical Research*, 124(1):95-98.

Hall-Stoodley, L., Costerton, J. W. and Stoodley, P. (2014). Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*, 2(2):95-108.

Hassan, A., Usman, J., Kaleem, F., Omalr, M., Khalid, A. and Iqbal, M. (2015). Evaluation of different detection methods of biofilm formation in clinical isolates. Brazilians Journal of Infectious Diseases, 15(4):305-311.

Hoang, T. H., Wertheim, H., Minh, N. B., Duong, T. N., Anh, D. D., Phuong, T. T. (2013). Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains containing New Delhi metallo-β-lactamase isolated from two patients in Vietnam. *Journal of Clinical Microbiology,* 51, 373–374.

Howard, A., O'Donoghue, M., Feeney, A. and Sleator, R. (2012). *Acinetobacter baumannii:* An emerging opportunistic pathogen, *Virulence*, 3(3): 243–250.

Jan, M. B., John, D. T. and Sentry A. (2002). High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY Antimicrobial Surveillance Program, 1998-1999. *Antimicrobial Agents and Chemotherapy*, 46(3): 879-881.

Jena, J., Debata, N.K. and Subudhi, E. (2013). Prevalence of extended-spectrum-beta-lactamase and metallo-beta-lactamase producing multi drug resistance Gram-negative bacteria from urinary isolates, *Indian Journal of Medical Microbiology*; 31(4):: 420-421

Jimenez-Guerra, G., Heras-Cañas, V., Gutierrez-Soto, M., Aznarte-Padial, M., Expósito-Ruiz, M., Navarro-Marí1, J. and Gutierrez-Fernandez, J. (2018).,Urinary tract infection by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: evolution of antimicrobial resistance and therapeutic alternatives, *Journal of Medical Microbiology*, 67:790–797.

Manoharan, A., Premalatha, K., Chatterjee, S., Mathai, D. (2011). Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility. *Indian Journal of Medical Microbiology.* 29(2):161- 164.

Navon-Venezia, S., Chmelnitsky, I., Leavitt, A., Schwaber, M.J., Schwartz, D. and Carmeli**,** Y. (2006). Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. *AntimicrobialAgents and Chemotherapy,*50**:**3098-3101.

Qadri, S.M.H., Akhter, J. and Lee, G.C. (1886). Etiology of ICU infections and antibiogram of the isolates at a referral center in Riyadh. *Saudi Pharmaceutical Journal*. 4:174–177

Rupp, M.E. and Archer, G.D. (1994). Coagulase-negative staphylococci: pathogens associated with medical progress. *Clinical Infectious Diseases*, 19: 231-245.

Sachdeva, R., Sharma, B. and Rajni, ' R. (2017). Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant *Pseudomonas aeruginosa Journal of Laboratory Physicians*, 9(4): 249–253.

Sadhana, C., Smita, W., Charan, D., and Khare, A.S. (2014). Antibiotic resistance pattern of *Pseudomonas aeruginosa* with special reference to imipenem and MβL production. *Indian Journal of Basic and Applied Medical Research*, 4(1):117–122.

Thompson, K.S. (2010). Extended spectrum β –Lactamase, AmpC, and

carbapenemases issues. *Journal of Clinical Microbiology*, 48:1019-1025.

Zubair, K.O. and Iregbu, K.C. (2018). Resistance pattern and detection of metallo-beta-lactamase genes in clinical isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital, *Nigerian Journal of Clinical Practice,* 21 (2):176-182.

Villegas, M. V., Lolans, K., Correa, A., Kattan, J.N., Lopez, J.A. and Quinn**,** J.P**.** (2007). First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. *Antimicrobial Agents and Chemotherapy,* 51:1553-1555.