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Characterization and Colistin Susceptibility of Carbapenem Resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from Various Clinical Specimens

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A B S T R A C T

Rising antimicrobial resistance is a major threat in the management of infections caused by *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Colistin is considered as last resort in management of these infections. This study aims to determine prevalence of carbapenem resistance in *P.aeruginosa* and *A.baumannii*, to characterize the prevalent carbapenem resistance mechanisms and to evaluate their in vitro colistin susceptibility. Materials and methods: 150 consecutive (75 *P.aeruginosa* and 75 *A.baumanni*) isolates were included. Antimicrobial susceptibility testing was performed as per CLSI guidelines. Imipenem MIC was determined by E-test. Carbapenemase production tested by Modified Hodge test and MBL detection. Genotypic confirmation was done by PCR. Colistin MIC was determined by E-test. Results: Prevalence of MDR among *P.aeruginosa* was 29.3% and among *A.baumannii* was 50.7%. Among *P.aeruginosa*, 5.3% were carbapenemase producer by MHT and all were found to be MBL by Imipenem-EDTA combined disc method. Among *A.baumannii*, 12% were positive for carbapenemase production by MHT; 4% were MBL producers. 4 of 19 carbapenem resistant isolates were having MIC in upper limit of susceptible range (1.5 to 2 µg/ml). Conclusion: Because carbapenem resistance is also associated with resistance to antibiotics of other classes, the therapeutic options are very limited. MIC testing should be performed for colistin.

Introduction

Antimicrobial resistance is on the rise and it is a major public health problem across the world, especially in developing countries like India. The continuing emergence of resistant strains causing nosocomial infections contributes to the morbidity and mortality among hospitalized patients. Of the nosocomial pathogens, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are of great concern for patients admitted in intensive care units [ICU]. (Shanthi and Uma Sekar, 2009). Management of these infections is difficult, as many strains often develop intrinsic and acquired resistance to multiple

classes of antimicrobial drugs. (Srujana Mohanty *et al.,* 2013) Various mechanisms for MDR include loss of outer membrane protein, overexpression of efflux pump, production of β- lactam hydrolyzing enzymes such as extended spectrum β-lactamases (ESBL) & AmpC β-lactamases and carbapenem hydrolyzing enzymes (metallo-β- lactamases, oxacillinase).

The introduction of carbapenem antibiotics such as meropenem and imipenem into clinical practice was of great help in the treatment of serious infections caused by the ESBL and AmpC producing multidrug-resistant (MDR) bacteria. (Srujana Mohanty *et al.,* 2013) However, the resistance to these drugs is also on the rise because of emergence of metallo β-lactamases (MBL) and OXA type carbapenemases, which is seen predominantly in *Acinetobacter baumannii*.

Globally, reports on the carbapenemase-producing non-fermenting Gram- negative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are on the rise due to the increased carbapenem usage and selection pressure. In India, carbapenem resistance ranges from 10.9 - 69% in *Pseudomonas aeruginosa* and 9.1-100% in *Acinetobacter baumannii* has been reported among various patient populations in different sample types, predominantly from respiratory specimens and pus samples. (Shashikala *et al.,* 2006; Behera *et al.,* 2008; Gaur *et al.,* 2008 and Uma Karthika *et al.,* 2009)

As the production of the carbapenem hydrolyzing enzyme is plasmid mediated, it limits the therapeutic options and is a matter of serious concern for infection control management.

Therefore, early identification and detection of isolates that produce these enzymes are essential to avoid therapeutic failures and nosocomial outbreaks. (Richa Gupta *et al.,* 2016) World Health Organization (WHO) (2017) has categorized carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as Priority 1- Critical organisms for the research and development of newer antibiotics.

Colistin (Polymyxin E), was one of the earliest polymyxin antibiotics, used for the treatment of gram-negative bacterial infections; however, side effects such as nephrotoxicity, and the development of less toxic antibiotics, led to its withdrawal from general use. The appearance of multidrug resistant strains of *A.baumannii* and *P. aeruginosa* has once again led to the reconsideration of colistin for the treatment of carbapenem resistant gram-negative bacterial infections. Susceptibility testing for colistin should be carried out prior to administration to prevent treatment failure.

There is enormous geographic variation in the prevalence of antimicrobial resistance; therefore the resistance profile of resistant strains requires enhanced monitoring, especially for selection of empirical antibiotic. Obtaining regional resistance data is important for formulating guidelines for appropriate antibiotic use, and may help to control the rate of antimicrobial resistance.

In this background this study aims to determine the prevalence of carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates among various clinical samples, to characterize the prevalent carbapenem resistance mechanisms phenotypically and genotypically and to evaluate the *in vitro* susceptibility of colistin against the carbapenem resistant isolates.

Materials and Methods

This descriptive study was conducted for one year at Institute of Microbiology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai-3. Approval was obtained from Institutional Ethics Committee. Statistical analysis was done by Pearson's chi-square test using SPSS software. 150 Clinically significant, consecutive, non-repetitive isolates of *Pseudomonas aeruginosa* (75 isolates) and *Acinetobacter baumannii* (75 isolates) from various clinical specimens were

included. The significance of the isolates was based on two or more of the following criteria – clinical history, presence of organism in Gram stain, presence of intracellular forms of the organism and pure growth in culture with a significant colony count wherever applicable. Isolates of repeated samples from the same patient were excluded. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates were phenotypically characterized based on culture, microscopy and biochemical reactions.

Antimicrobial sensitivity testing was performed for all the isolates by Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates against Cephalosporins, β-lactam/β-lactamase inhibitors, Carbapenems, Fluoroquinolones, Aminoglycosides according to CLSI guidelines 2017. MIC of Imipenem was determined by Epsilometer test (E-test) for all the imipenem intermediate and resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

According to CDC-NHSN, isolates showing resistance to at least one agent in three or four groups of antibiotics (cephalosporins, carbapenems, fluoroquinolones and aminoglycosides) were considered as Multi-Drug Resistant (MDR) in this study.

Detection of antimicrobial resistance Phenotypic screening methods

All the isolates included were subjected to carbapenemase screening test using imipenem (10µg) and meropenem (10µg) discs. The isolates which were positive in the screening test were subjected to the phenotypic confirmatory tests.

Carbapenemase detection by Modified Hodge Test (MHT) (Gniadek *et al.,* 2016; Lee *et al.,* 2001)

Isolates positive for screening test in the disc diffusion (resistant to carbapenems) was further processed by modified Hodge test to detect carbapenemase production according to CLSI 2017.

Metallo beta-lactamase (MBL) detection by combined disc method

Metallo-β-Lactamase production for the carbapenem resistant isolates was screened by imipenem (10µg) –EDTA (750µg), meropenem (10µg) – EDTA (930µg) and ceftazidime (30µg) –EDTA (930µg) combined disc method. An increase in zone size of ≥ 7 mm around Inhibitor combination disc compared to disc without inhibitor was considered as MBL positive. (Manoharan *et al.,* 2010)

Colistin MIC was determined for carbapenem resistant isolates by Epsilometer test (E-test) using colistin E-strips of concentration gradient (0.016 to 256µg/ml (Biomerieux).

Molecular characterization

The Carbapenem resistant *Pseudomonas aeruginosa* isolates which were positive for MBL production and carbapenem resistant *Acinetobacter baumannii* isolates which were positive for carbapenemase production by modified Hodge test were subjected to conventional Polymerase Chain Reaction (PCR) for the detection *bla*VIM/*bla*NDM-1 and *bla*OXA-23 genes respectively.

Results and Discussion

In this descriptive study, 75 clinically significant, consecutive, non- repetitive *Pseudomonas aeruginosa* and 75 *Acinetobacter baumannii* isolates collected from various clinical specimens were included.

Currently, antimicrobial resistance especially for carbapenem is a major public health issue which increases morbidity and mortality in hospitalized patients.

Since plasmid mediated carbapenemase production is the major cause of dissemination of antimicrobial resistance genes between various bacterial species, identifying the prevalence and mechanism of carbapenem resistance and their susceptibility to other antibiotics are necessary to formulate

antibiotic policies in a hospital and to determine various treatment options.

In this study, the majority of *Pseudomonas aeruginosa* isolates were from pus sample – post operative wound and diabetic foot (42.7%), followed by respiratory specimens (33.3%). Similarly, *Acinetobacter baumannii* isolates were predominantly isolated from respiratory specimens (29.3%) followed by pus sample (28%) – Chart 1. In a study conducted by *Shasikala et al.,* (2006) at Pondicherry, 27.6% of the *P.aeruginosa* isolates were from wound infections. Padmalakshmi *et al.,* (2015) reported 37.5% of *A.baumannii* isolates were from respiratory specimen which is similar to this study. Both *P.aeruginosa* and *A.baumannii* are ubiquitous, can tolerate harsh environments and hence, colonizes the skin and respiratory tract more commonly in hospitalized patients.

The overall resistance of *P.aeruginosa* vs *A.baumannii* to the antibiotics tested was ceftazidime (65.3% vs 81.3%), pipercillin-tazobactam (30.7% vs 36%), amikacin (16% vs 32%), gentamicin (36% vs 70.1%), ciprofloxacin (40% vs 53.3%), meropenem (12% vs 13.3%) and imipenem (5.3% vs 13.3%) – Table 1 & 2.

Mohanty et al., in their study at New Delhi found that the overall resistance of the isolates (*P. aeruginosa* vs *Acinetobacter* spp.) to the antibiotics was ceftazidime (57.9% vs 84.0%), piperacillin/tazobactam (22.1% vs 42.0%), amikacin (33.7% vs 72.0%), gentamicin (40.0% vs 80.0%), ciprofloxacin (35.8% vs 64.0%), meropenem (36.8% vs 62.0%) and imipenem (37.9% vs 64.0%). (Srujana Mohanty *et al.,* 2013) Benachinmardi Kirtilaxmi *et al.,* (2014) in a recent study at PGIMSR, Bengaluru showed 80% of *P.aeruginosa* and about 41% *A.baumannii* were sensitive to imipenem.

According to the study conducted at Trichy in 2015, 8.7% of NFGNB were resistant to meropenem. (Jane Esther *et al.,* 2017) This is concordant with the present study which shows about 12% carbapenem resistance in *P.aeruginosa* and 13.3% in *A.baumannii*. These differences in the antimicrobial susceptibility could be due to the geographical variation. Therefore, various international authorities emphasize that every hospital should have its own antibiotic policy.

In this study, the prevalence of MDR among *P.aeruginosa* was found to be 29.3% and among *A.baumannii* was 50.7% (Table 3). This is similar to the study conducted in Italy by *Franesco De et al.,* (2013) who showed the prevalence of MDR among *P.aeruginosa* and *A.baumannii* as 20% and 54% respectively. Khan *et al.,* (2014) reported 30% of *P.aeruginosa* isolates as MDR, while Lakshmi Vemu *et al.,* (2014) found 77% of *A.baumannii* to be multidrug resistant.

Among *P.aeruginosa*, 12% were carbapenem resistant, 5.3% were positive for carbapenemase production by Modified Hodge test and all these were found to be Metalloβ-lactamase by Imipenem-EDTA combined disc method; while among *A.baumannii* 13% were carbapenem resistant, 12% were positive for carbapenemase production by Modified Hodge test and 4% were metalloβ-lactamase producers (Table 4). According to the study of *Gupta et al.,* (2016) among non-fermenters 21.4% were metallobeta- lactamase producers.

Five of the nine meropenem resistant *P.aeruginosa* isolates were sensitive to Imipenem; they were categorized as MRIS (Meropenem Resistant Imipenem Sensitive). In MRIS phenotype, the carbapenem resistance could be due to over expression of efflux pump which can be confirmed by the genotypic methods. In *A.baumannii*, all the carbapenem resistant isolates were resistant to both meropenem and imipenem; they belong to IRMR (Imipenem Resistant Meropenem Resistant) phenotype in which the carbapenem resistance is predominantly enzyme mediated (carbapenem hydrolyzing enzymes such as OXA type carbapenemases and Ambler class B metalloβlactamases) (Agila *et al.,* 2016).

The emergence of these phenotypes occurs mainly due to the antibiotic selection pressures due to inappropriate dosage and duration of the carbapenems. Hence, it is advisable to perform antimicrobial susceptibility testing for each of the carbapenems namely imipenem, meropenem and doripenem, rather than testing single carbapenem and to extrapolate the results for other carbapenems.

Out of four MBL producing *P.aeruginosa*, two isolates were positive for *bla*NDM-1 gene by conventional PCR, one isolate was positive for *bla*VIM gene and one was negative for both; five carbapenem resistant *P.aeruginosa* isolates were MRIS phenotypes which could be due to over expression of efflux pumps. Of the nine carbapenemase producing *A.baumannii*, four were positive for *bla*OXA-23 gene and three were found to be metalloβ-lactamase producers by phenotypic method. Therefore the predominant mechanism for carbapenem resistance among *P.aeruginosa* and *A.baumannii* was efflux pump over expression and carbapenemase production respectively.

In *P.aeruginosa*, carbapenem resistant isolates were equally distributed between respiratory specimen especially endotracheal aspirate and urine (44.4%) whereas in *A.baumannii*, carbapenem resistance was noted predominantly among respiratory specimen (endotracheal aspirate - 50% & sputum 10%) as most of these isolates were from ICU patients on mechanical ventilation (Table 5).

Of the total 19 carbapenem resistant isolates (nine *P.aeruginosa* and ten *A. baumannii*), 52.6% were isolated from patients admitted in Intensive care units (Table 7). This correlates with the studies conducted in Iran and New Delhi which reported 53.6% and 67.5% carbapenem resistance among patients admitted in intensive care unit respectively. (Tempe *et al.,* 2015 and Mahtab Noorifard *et al.,* 2016) This high carbapenem resistance rate in ICU is due to the associated risk factors such as prolonged hospital stay, interventions such as mechanical ventilation and previous use of antibiotics especially carbapenem.

This increase in carbapenem resistance in ICU is alarming and hence it is necessary to take various preventive measures which include screening for carbapenem resistance carriers in high risk units (surveillance cultures), undertaking strict contact precautions for carriers and antibiotic stewardship programs to spare carbapenems.

Among the 14 imipenem resistant isolates (four *P.aeruginosa* and ten *baumannii*), three isolates showed MIC of imipenem in the intermediate range (Table 6). Recent studies showed that extended infusion therapy of carbapenem for about 30 minutes to 3 hours was found to be effective if the MIC of carbapenem falls in the intermediate range (4µg/ml to <8µg/ml). (Peleg *et al.,* 2008; Kanj and Kanafani, 2011 and Manchanda *et al.,* 2010) Hence, mere screening for carbapenem susceptibility is insufficient and detection of MIC is essential as it can determine the appropriate treatment regimens.

Carbapenem resistant *P.aeruginosa* isolates were also 100% resistant to ceftazidime, cefepime and piperacillin-tazobactam; however they were highly sensitive to amikacin (55.6%). Similarly, carbapenem resistant *A.baumannii* isolates were 100% resistant to ceftazidime, piperacillin-tazobactam, gentamicin and trimethoprim-sulfamethoxazole (Table 8&9). This is statistically significant ($p<0.05$) and in parallel to the study by Srujana Mohanty *et al.,* (2013)

Since carbapenem resistance is predominantly mediated by multi-drug resistance transferrable plasmids, carbapenem resistant strains remain resistant to several other antibiotics including fluoroquinolones, aminoglycosides, third generation cephalosporins such as ceftazidime and β-lactam/β-lactamase inhibitor combinations. This poses serious problems in choosing the right antibiotic for the treatment of hospitalized patients admitted in ICU.

Table.1 Antimicrobial Susceptibility pattern of *Pseudomonas aeruginosa* isolates (n=75)

Antimicrobial agent	No. of susceptible isolates (%)	No. of resistant isolates (%)
Ceftazidime(30µg)	26(34.7%)	**49(65.3%)**
Piperacillin-Tazobactam(100/10µg)	52(69.3%)	**23(30.7%)**
Gentamicin(10µg)	48(64%)	**27(36%)**
Cefepime(30µg)	42(56%)	**33(44%)**
Amikacin(30µg)	63(84%)	**12(16%)**
Ciprofloxacin(5µg)	45(60%)	**30(40%)**
Meropenem(10µg)	66(88%)	**9(12%)**
Imipenem(10µg)	**71(94.7%)**	**4(5.3%)**

Table.2 Antimicrobial Susceptibility pattern of *Acinetobacter baumannii* isolates (n=75)

Antimicrobial agent	No. of susceptible isolates (%)	No. of resistant isolates (%)
Ceftazidime(30µg)	14(18.7%)	**61(81.3%)**
Ciprofloxacin(5µg)	35(46.7%)	**40(53.3%)**
Gentamicin(10µg)	22(29.3%)	**53(70.1%)**
Meropenem(10µg)	65(86.7%)	**10(13.3%)**
Imipenem(10µg)	65(86.7%)	**10(13.3%)**
Piperacillin-Tazobactam(100/10µg)	48(64%)	**27(36%)**
Amikacin(30µg)	51(68%)	**24(32%)**
Trimethoprim-sulfamethoxazole(1.25/23.75µg)	14(18.7%)	**61(81.3%)**
Tetracycline(30µg)	**42(56%)**	**33(44%)**

Table.3 Distribution of Multidrug Resistance and Carbapenem resistance among *P.aeruginosa* and *A.baumannii* isolates

Organism	No. of isolates		
	Total isolates(n)	MDR(%)	Carbapenem resistant(%)
Pseudomonas aeruginosa	75	22(29.3%)	**9(12%)**
Acinetobacter baumannii	**75**	**38(50.7%)**	**10(13%)**

MDR–Multidrug resistant

Table.4 Phenotypic characterization of resistance among *Pseudomonas aeruginosa* isolates (n=75) & *Acinetobacter baumannii* isolates (n=75)

Phenotypic tests	No. of *Pseudomonas aeruginosa* isolates positive(%)	No. of *Acinetobacter baumannii* isolates positive(%)
Carbapenem resistant (by disc diffusion)	9(12%)	**10(13.3%)**
Carbapenemase production by Modified Hodgetest	4(5.3%)	**9(12%)**
MBL detection	**4(5.3%)**	**3(4%)**

Table.5 Distribution of Carbapenem resistant *P.aeruginosa* and *A.baumannii* isolates among various samples

Clinical samples	No. of Carbapenem resistant *P.aeruginosa* (n=9)	No. of Carbapenem resistant *A.baumannii* (n=10)
Respiratory specimens	4(44.4%)	**6(60%)**
Urine	4(44.4%)	**1(10%)**
Blood	-	**3(30%)**
Fluids	1(11.1%)	-
***p value**	**0.006**	**0.007**

*p<0.05–Statistically significant

Table.6 Minimum Inhibitory Concentration of Imipenem for the Imipenem resistant isolates by disc diffusion method (n=14)

Organism	No. of isolates with Minimum Inhibitory Concentration(MIC)-µg/ml		
	≤2Sensitive	4-8Intermediate	≥8Resistant
***P.aeruginosa* (n=4)**	-	1	**3**
***A.baumannii* (n=10)**	-	**2**	**8**

Table.7 Risk factors associated with carbapenem resistance (n=19)

Risk factors	Occurrence(%)
Hospital stay>7days	**14(73.7%)**
Mechanical Ventilation	**12(63.2%)**
ICU admission	**10(52.6%)**
Urinary catheterization	**18(94.7%)**
Previous use of antibiotic (Carbapenem)	**5(26.3%)**
Previous surgery	**3(15.8%)**

Mortality–6(31.2%)

Table.8 Antimicrobial resistance pattern among Carbapenem susceptible and Carbapenem resistant *P.aeruginosa* isolates

Antibiotic	No. (%) of resistant isolates among			
	Total(n=75)	Carbapenem susceptible isolates (n=66)	Carbapenem resistant isolates (n=9)	*p value
Ceftazidime(30µg)	49(65.3%)	40(60.6%)	9(100%)	**0.023**
Cefepime(30µg)	33(44%)	24(36.4%)	9(100%)	**0.0001**
Piperacillin-Tazobactam(100/10µg)	23(30.7%)	14(21.2%)	9(100%)	**0.0001**
Amikacin(30µg)	12(16%)	8(12.1%)	4(44.4%)	**0.032**
Gentamicin(10µg)	27(36%)	19(28.8%)	8(88.8%)	**0.001**
Ciprofloxacin(5µg)	**30(40%)**	**22(33.3%)**	**8(88.8%)**	**0.002**

Table.9 Antimicrobial resistance pattern among Carbapenem susceptible and Carbapenem resistant *A.baumannii* isolates

Antibiotic	No. (%) of resistant isolates among			
	Total (n=75)	Carbapenem susceptible isolates (n=65)	Carbapenem resistant isolates (n=10)	*p value
Ceftazidime(30µg)	61(81.3%)	51(78.5%)	10(100%)	**0.109**
Piperacillin-Tazobactam(100/10µg)	27(36%)	17(26.2%)	10(100%)	**0.0001**
Amikacin(30µg)	24(32%)	16(24.6%)	8(80%)	**0.001**
Gentamicin(10µg)	53(70.1%)	43(66.2%)	10(100%)	**0.024**
Ciprofloxacin(5µg)	40(53.3%)	32(49.2%)	8(80%	**0.068**
Trimethoprim-Sulfamethoxazole (1.25/23.75µg)	61(81.3%)	51(78.5%)	10(100%)	**0.109**
Tetracycline(30µg)	**33(44%)**	**25(38.5%)**	**8(80%)**	**0.09**

Table.10 MIC of Colistin for the Carbapenem resistant isolates (n=19)

Organism	No. of isolates with Minimum Inhibitory Concentration (MIC)-µg/ml			
	Sensitive			Resistant
	≤0.5	0.5–1	1–2	≥4
***P.aeruginosa* (n=9)**	2	4	3	-
***A.baumannii* (n=10)**	**5**	**4**	**1**	-

Chart.1 Sample wise distribution of *P.aeruginosa* and *A.baumannii*

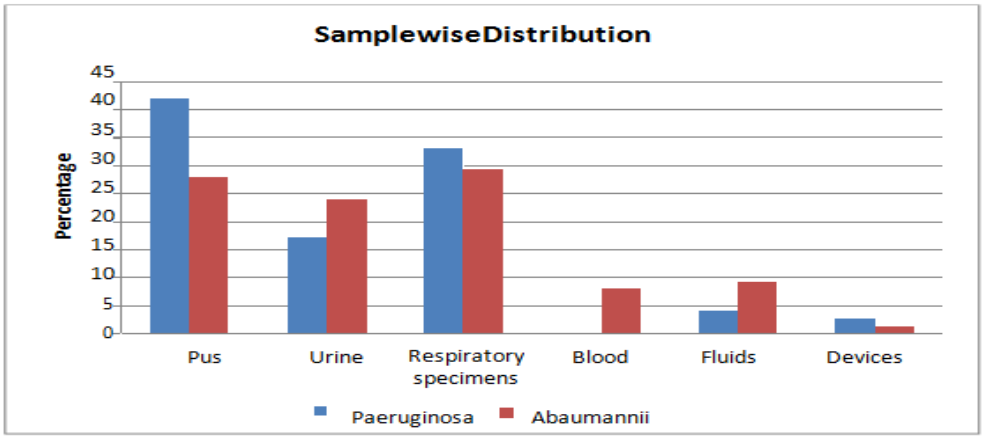


Fig.1 MRIS–Meropenem Resistant Imipenem Sensitive phenotype of Carbapenem resistance in *Pseudomonas aeruginosa*

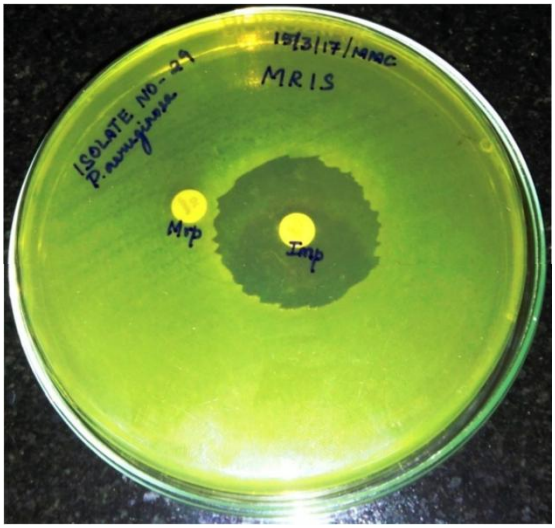


Fig.2 Modified Hodge Test (MHT) A54 & P29MHT positive for carbapenemase production. Arrow shows Clover-leaf indentation

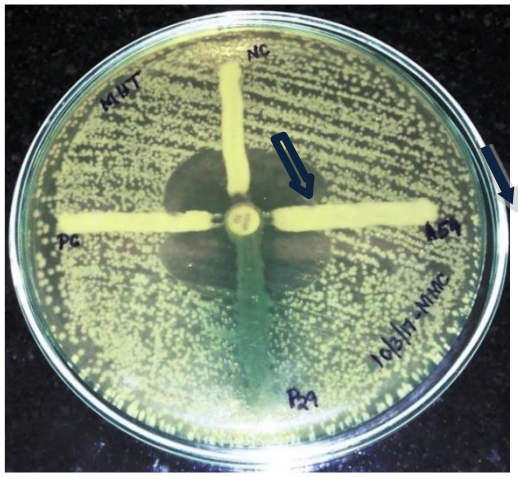
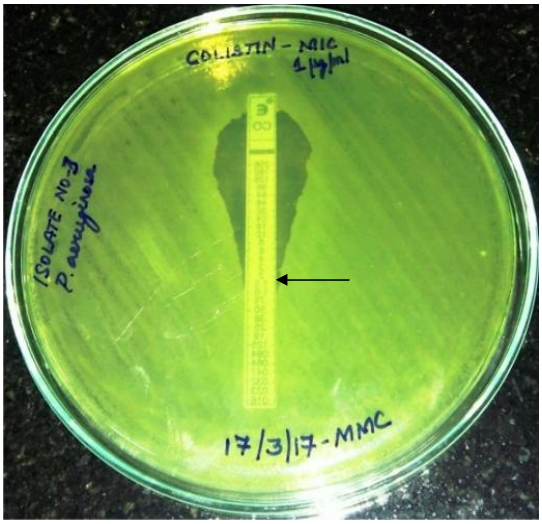


Fig.3 Metallo Beta-Lactamase (MBL) detection by Combined disc method using Imipenem with Imipenem-EDTA(10/750µg/ml), Meropenem with Meropenem-EDTA(10/930µg/ml) & Ceftazidime with Ceftazidime-EDTA(10/930µg/ml)

Fig.4 Colistin MIC by E-test for Carbapenem resistant *Pseudomonas aeruginosa* showing MIC of 1µg/ml (colistin sensitive)



All the 19 carbapenem resistant isolates were 100% susceptible to colistin. Baurah *et al.*, (2015) in a study in 2014 reported that *P.aeruginosa* was 100% susceptible to Colistin. However according to Srujana Mohanty *et al.*, (2013) in India, the prevalence of colistin resistance was found to be 6% in *A.baumannii* and about 8% in *P.aeruginosa* which is in contrast to this study. Taneja *et al.*, (2011) also reported about 3.5 % of *A.baumannii* to be colistin resistant. This variation in the prevalence of colistin resistance could be due to the geographic variation and different antibiotic policies among various hospitals.

Four of the 19 carbapenem resistant isolates were having MIC in the upper limit of susceptible range (1.5 to 2 µg/ml) – Table 10; this indicates that the MIC testing for colistin should be made mandatory

before administration to prevent the emergence of colistin resistance in a community, as colistin is the only available effective antibiotic for the treatment of carbapenem resistant infections.

In conclusion, Carbapenem resistance is increasing in the post-antibiotic era under the selection pressure of carbapenem in clinical settings. Detection of carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* has great impact on hospital infection control and for epidemiological purpose for the prevention of further spread of resistance in the community. To overcome the resistance, implementation of strict infection control practices and active surveillance of genes encoding carbapenemase are necessary.

Because carbapenem resistance is also associated with resistance to antibiotics of other classes, the therapeutic options are very limited. Although colistin may be considered as an alternative for infections caused by carbapenem- resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, MIC testing should be performed whenever clinical use of colistin is considered to contain the emerging colistin resistance.

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