OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/sjpm/

Original Research Article

Detection and Antibiotic Susceptibility Pattern of Pseudomonas Aeruginosa Isolates from Various Clinical Samples in Tertiary Care Hospital, Bhavnagar, Gujarat

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| **Received:** 19.03.2019 | **Accepted:** 27.03.2019 | **Published:** 31.03.2019
DOI:10.21276/sjpm.2019.4.3.10

Abstract

Introduction: Pseudomonas aeruginosa has been emerged as an important opportunistic pathogen. Being an extremely adaptable organism, it can survive and multiply even with minimal nutrients and is one of the leading causes of hospital acquired infections. P. aeruginosa exhibits intrinsic resistance to several antimicrobial agents. As a result of indiscriminate use of antibiotics, the spread of multidrug resistance (MDR) is now a global problem. Its general resistance is due to a combination of factor.Emergence of carbapenem resistance mainly Metallo-Beta-Lactamase (MBLs) in Pseudomonas aeruginosa which is considered as a world wide public health concern. ***Objectives:*** To study the detection of Pseudomonas aeruginosa and its antibiotic susceptibility pattern from various clinical samples in Tertiary Care Hospital, Bhavnagar, Gujarat. ***Materials and Methods:*** The Present study was undertaken at Microbiology Laboratory, Sir T. Hospital, Bhavnagar. 300 isolates of Pseudomonas aeruginosa were collected from various clinical samples between November-2013 to August-2014 .They were subjected to antibiotic sensitivity testing by Modified Kirby Bauer Disc Diffusion Method as per CLSI guidelines. Quality control of the test was done by standards ATCC strain P. aeruginosa 27853. ***Results:*** 300 Isolates were included in the study, out of which 95(32%) showed Imipenem Resistant. and were 100% resistant to Cefoatxime, and Imipenem. Gentamicin and Ciprofloxacin showed 84% and 93% resistance. Amikacin and Ofloxacin showed 89% resistance each. where as Piperacillin showed 75% resistance. ***Conclusions:*** Early detection will go a long way in making adjustments in empirical antimicrobial therapy. The study was coducted to formulate antibiotic policy and plan a proper hospital infection control strategy to prevent the spread of these MDR strains.

Keywords: Pseudomonas, Antibiotic sensitivity, Imipenem, Multidrug Resistance.

INTRODUCTION

Pseudomonas aeruginosa, an opportunistic and worrisome nosocomial pathogen, is a Gram-negative, slender, aerobic rod, 1.5-3x0.5µm belonging to bacterial family Pseudomonadaceae [1, 2]. It is actively motile by polar flagella, non sporing, non capsulated but mucoid strains have extracellular polysaccharide composed of alginate polymers and are often pilated. Many have mucoid slime layer [3].

It is the pseudomonad most frequently recovered from clinical specimens. It is reported to be a leading cause of nosocomial infections, including pneumonia, urinary tract infection, burn infection, meningitis and bacteraemia [2]. P. aeruginosa accounts for about 6% of nosocomial bacteremias [4] and is the third most common cause of bacteremia, after Esherichia coli and Klebsiella pneumoniae [1].

Its ability to survive on inert materials, live on minimal nutritional requirement, with its tolerance to a wide variety of physical conditions and antiseptics [2]. P. aeruginosa prefers moist environments, and in hospitals they can be isolated from nebulizers, dialysate fluids, saline, on catheters and other diagnostic and therapeutic devices. They can grow in distilled water using, dissolved carbon dioxide and residual sulfur, phosphorus, iron and divalent cations as carbon and essential nutritional substrates. It can withstand treatment with chlorohexidine and quaternary ammonium compounds. They are rarely if ever, part of the normal flora but can easily colonize hospitalized

patients especially those who are immunocompromised [5].

It is Killed at 55°c in 1hour. It is resistant to common antiseptics and disinfectants such as quarternary ammonium compounds, chloroxylenol, dettol and hexachlorophene. It may grow profusely in bottles of such antiseptic lotion kept for use in hospitals for preservation of ophthalmic solutions. It is susceptible to acids, β-glutaraldehyde, silver salts and phenolic disinfectant. It is susceptible to silver compounds, hence silver compounds are used as topical cream in burns applied clinically [3]. These Characteristics have contributed enormously to its ecological success and its role as an effective opportunistic pathogen.

Pseudomonas aeruginosa has one of the broadest ranges of infectivity amongst all pathogenic microorganisms [6]. Infections caused by P. aeruginosa are more dangerous because the organism is inherently resistant to many antibiotics and is able to acquire resistance to all effective antimicrobial drugs [7].

Its general resistance is due to a combination of factors like:

- It is intrinsically resistant to antimicrobial agents due to low permeability of its cell wall.
- It has the genetic capacity to express a wide range of resistance mechanisms.
- It can become resistant through mutation in chromosomal genes which regulate resistance genes.
- It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages [8].
- It produces enzymes namely ß-lactamases, which are responsible for wide spread ß-lactam resistance [9].

Biofilm Formation

P.aeruginosa has high intrinsic resistance to many antibiotics at levels attainable in body tissues. A narrow spectrum of effective antimicrobials for P.aeruginosa are Carboxypencillins (Carbenicillin, Ticarcillin), Ureidopencillins (Mezlocillin, Piperacilli), antipseudomonal cephalosporins (Ceftazidime), Monobactums (Aztreonam), Carbapenems (imipenem,meropenem), Quinolones (Ciproflaxacin, Levofloxacin) and Aminoglycosides (Gentamicin, Tobramycin, Amikacin). The Carbapenems are currently among the last resort for the treatment of serious MDR Pseudomonas aeruginosa infection [10].

P.aeruginosa represents a phenomeneon of antibiotic resistance, and demonstrates practically all known enzymic and mutational mechanisms of bacterial resistance. It is intrinsically resistant to many structurally unrelated antimicrobial agents due to low permeability of its outer membrane, the constitutive expression of various efflux pumps with wide substrate specificity and naturally occurring chromosomal AmpC β- lacatamse. Naturally resistant of pseudomonas relates to Penicillin G, Aminopenicillins, first and second generation cephalosporins [11].

In P.aeruginosa all possible mechanisms determining resistance to β-lactam antibiotics (enzymatic inactivation, active efflux, changes in outer membrane permeability and synthesis of penicillin binding properties with lower affinity to β-lactams) may exist simultaneously or in various combinations [11].

Further, as the genes coding them are carried on highly mobile elements, their spread in recent years from P. aeruginosa to Enterobacteriaceae has lead to a situation where a clinical scenario that could simulate the global spread of ESBL appears to be developing [12, 13].

In India prevalence of MBLs production in P. aeruginosa varies from one region to another and range is from 7 – 65% [14, 15]. The occurrence of an MBL positive isolate in a hospital environment poses not only a therapeutic problem, but is also a serious concern for infection control management [16] With the global increase in the occurrence and types of MBLs, early detection is crucial; the benefits of which include timely implementation of strict infection control practices and treatment with alternative antimicrobials [16, 17]. There is a difficulty in detecting such organisms which pose significant risks, particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBL gene transfer with other pathogenic hospital-related organisms, in the hospital [16, 18].

Clearly, in the absence of novel agents in the near future, the spread of MBL producers may lead to therapeutic dead ends. Early detection may avoid spread of these multidrug-resistant isolates and may help maintain first- and second-line therapies.

The severity of Pseudomonas aeruginosa infection can be limited by early detection and aggressive antibiotic treatment before the bacteria convert to a mucoid phenotype, but it is extremely difficult to eradicate once established [10].

Hence the present study is conducted for the isolation and identification of Pseudomonas aeruginosa, and its antibiotic susceptibility pattern to formulate antibiotic policy and plan a proper hospital infection control strategy to prevent the spread of these strains.

OBJECTIVES

To study the detection of Pseudomonas aeruginosa and its antibiotic susceptibility pattern from

various clinical samples in Tertiary Care Hospital, Bhavnagar ,Gujarat.

MATERIALS AND METHODS

In the present study ,300 isolates of Pseudomonas aeruginosa were obtained from various clinical specimens like pus, urine, burn, wound, sputum, body fluids (ascitic fluid, pleural fluid etc.), CSF, tracheal secretions, devices associated with patient (urinary catheter, endotracheal tube, i.v. catheter, tracheostomy tube etc.) from Sir Takhtsinhji General Hospital, Bhavnagar. The study period was from November 2013 to August 2014.These isolates were studied for their Antiobiotic susceptibility pattern.

All samples were collected under aseptic precautions by standard procedures and processed according to standard guidelines.

- Direct smear study: Direct smears with gram stain were screened for the presence of inflammatory cells and type of microbial flora. Gram stained smear shows gram negative bacilli along with pus cells.
- Specimens were inoculated on blood agar and Mac Conkey's agar plate, then incubated at 37 ^{0}C for 24 hours.
- Brain Heart Infusion broth was used for blood culture. The bottle was examined duly for turbidity and subculture was made at regular intervals on to blood agar, Mac Conkey's agar and any growth was processed further for identification.
- Identifiation of Pseudomonas aeruginosa
  a) Culture on blood agar yield flat irregular colonies with β-hemolysis.
  b) Non lactose fermenting colonies: Irregular, flat colonies on Mac Conkey's agar is seen.
  c) Colonies have characteristic fruity odour.
  d) Colonies were further identified by the following biochemical reactions. P.aeruginosa shows following results.

Test	Result
Catalase	+
Oxidase	+
Nitrate reduction	+
Indole	-
Citrate	+
Urease	Negative or weak positive
H2S production	-
TSI	Alkaline slant/no change
Arginine hydrolase	Positive
Mannitol fermentation	-
Sucrose fermentation	-

- Antiobiotic susceptibility testing: The isolates were subjected for antibiotic susceptibility testing by employing Kirby Bauer disc diffusion techniques according to CLSI guidelines. In the present study susceptibility was tested against following antibiotics procured commercially from Hi-media laboratories Ltd, Mumbai. The diameter of the zone was measured and interpreted according to the guidelines of CLSI.

Antibiotic discs	Concentration in µg	Resistant zone in mm	Intermediate zone in mm	Sensitive zone in mm
Ampicillin/Sulbactam(AS)	20	11	12-14	15
Co-Trimoxazole(BA)	25	10	11-15	16
Tetracyclin (TE)	30	14	15-18	19
Ceftizoxime (CI)	30	14	15-19	20
Cefotaxime(CF)	30	14	15-22	23
Ciprofloxacin(RC)	5	15	16-20	21
Piperacillin (PC)	100	17	-	18
Gentamicin(GM)	10	12	13-14	15
Amikacin(Ak)	30	14	15-16	17
Chloramphenicol (CH)	30	12	13-17	18
Ofloxacin(ZN)	5	12	13-15	16
Imipenem(I)	10	>16	13-16	<13

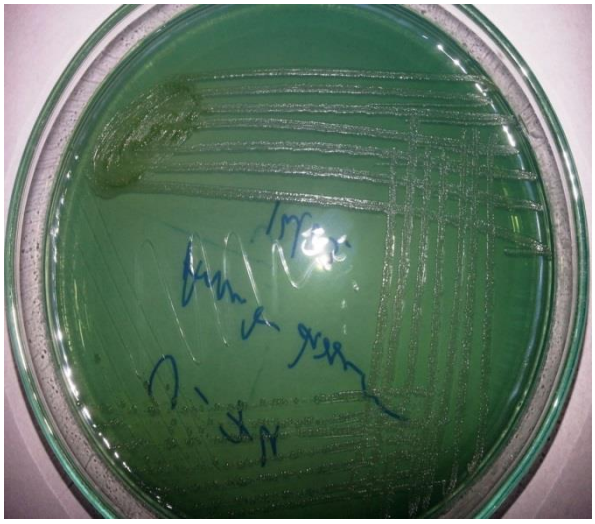


Fig-1: Bluish green pigmented colonies of p.aeruginosa on NA

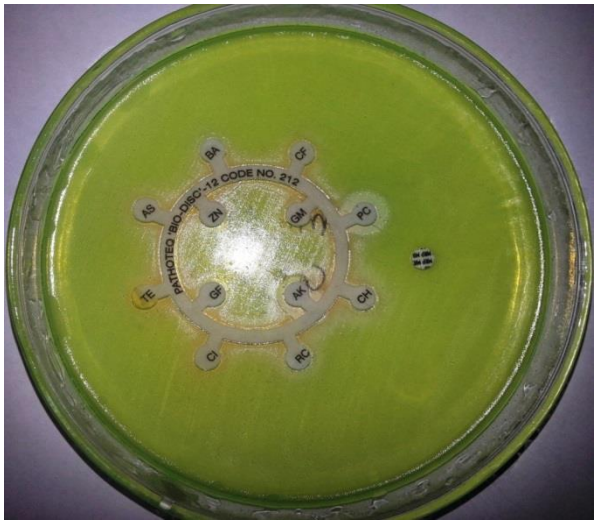


Fig-2: Multidrug resistant Pseudomonas isolate with fluorescence pigment

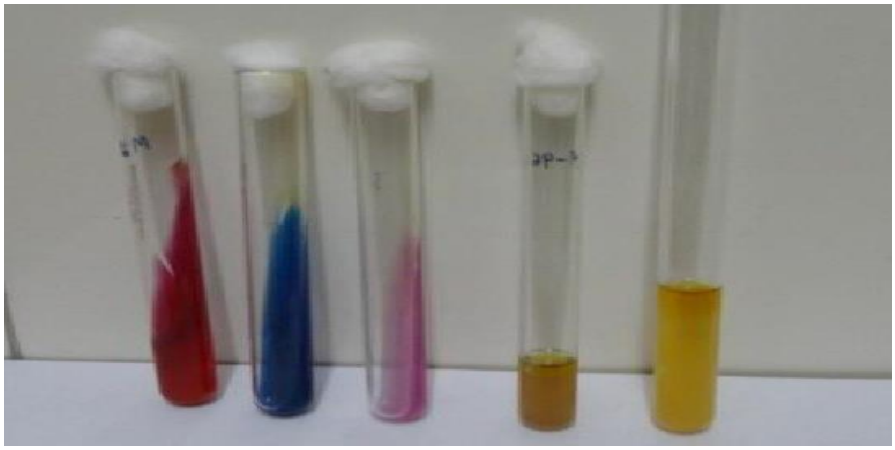


Fig-3: Biochemical Reactions of Pseudomonas

OBSERVATION & RESULTS

In the present study, 300 Pseudomonas aeruginosa isolates identified from various clinical samples like pus, urine, wounds, sputum, CSF, Body fluids (Ascitic fluid, pleural fluid etc.) during period of November 2013 to August 2014 at microbiology laboratory, Sir T. Hospital , Bhavnagar. These isolates were studied for antibiotic sensitivity.

Table-1: Age wise distribution of cases

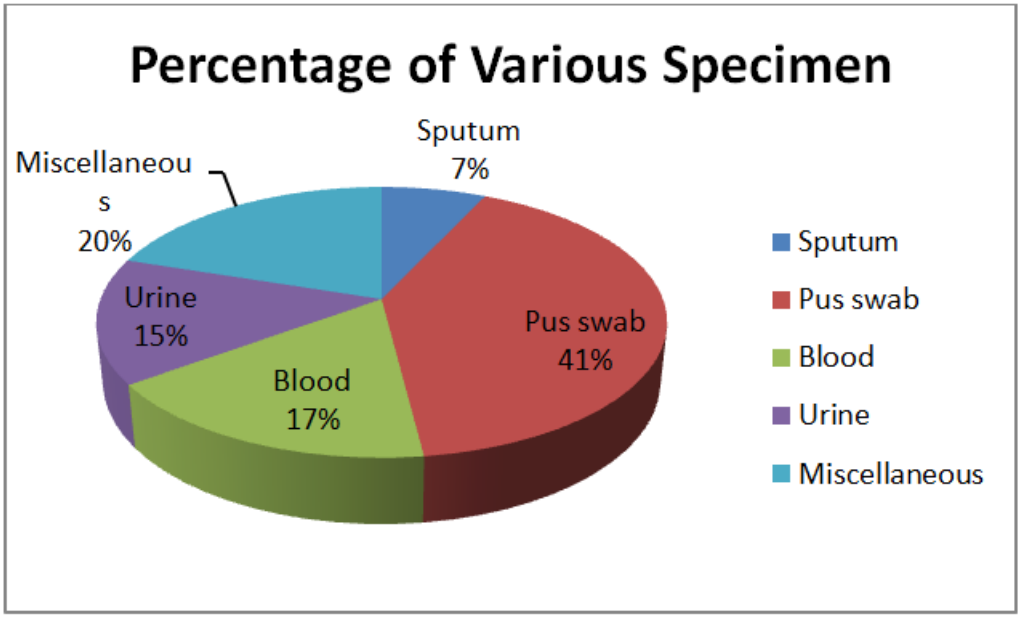
Age(years)	Number of cases	Percentage %
<10y	47	16
11 – 20	20	06
21 – 30	51	17
31 – 40	35	12
41-50	54	18
51-60	52	17
61-70	27	09
71-80	11	04
>80y	03	01
Total	300	100%

Age of the subjects in the Study group varies from 1 year to 80 years. Maximum number of isolates (54) were from the age group of 41-50years followed by 51-60years age group (52) and 21-30years age group (51) and least number of isolates (3) from more than 80years.

Table-2: Sex wise distribution of cases

Sex	No. of cases	Percentage%
Male	195	65
Female	105	35
Total	300	100%

Of the total number of samples, males constituted significant number of samples (65%). Male: Female is 1.85:1.



Graph-1: Showing Distribution of various specimens included in study

Total Pus swabs constituted majority of specimens accounting for 41%, urine and blood samples accounted for 15% and 17% respectively. Sputum and Miscellaneous (other body fluids) accounted for remaining 07% & 20%

Table-3: Distribution of cases in various wards

Wards	No. of cases	Percentage %
Surgical ward	**144**	**48**
Medical ward	**122**	**41**
ICCU	**34**	**11**

Total	**300**	**100%**

Of the total samples, highest number of samples 144(48%) were from Surgical wards (surgery, Orthopedics, OBG, ENT) followed by 122(41%) from Medical wards (Medicine and Pediatrics) and remaining 34(11%) were from ICCU.

Table-4: Resistance pattern of P.aeruginosa

Antibiotic	No.of isolates	Percentage%
Ampicillin+sulbactam(AS)	174	58
Cotrimoxazole (BA)	185	52
Cefotaxime (CF)	216	72
Ceftazidime(CAZ)	267	89
Piperacillin (PC)	84	28
Chloramphenicol (CH)	180	60
Ciprofloxacin (RC)	171	57
Ceftizoxime (CI)	74	25
Tetracycline (TE)	207	69
Ofloxacin (OF)	120	40
Gentamicin (GM)	135	45
Amikacin (AK)	120	40
Imipenem (IMP)	95	32

72% of the isolates were resistant to Cefotaxime. Among aminoglyosides least resistance was shown by Amikacin 40% and highest by Gentamicin 45%. While resistance to Imipenem was noted in 32%, Piperacillin showed 28% resistance and Chloramphenicol showed 60% resistance. Prevalence of MBL production was seen in 18% of isolates.

DISCUSSION

In the present study, an attempt was made to know the isolation of P. aeruginosa and also their antibiotic sensitivity pattern at Microbiology Laboratory, Sir T. Hospital, Bhavnagar. The results are compared with other studies. Factors such as age and sex among MDRPA infection were found to have more significant association with isolation of MDRPA [19].

Table-1: Age wise distribution in various studies.

Study series	Year	Age group affected	Percentage
Viren Javiya *et al.*, [20]	2006	21-60y	61.6
Rakesh R M *et al.*, [21]	2010	31-45y	29
Present study	2014	21-60y	64

In present study the age wise prevalence of clinical isolates shows that most of patients 64% were aged between 21-60 years. This is in accordance with study of Viren Javiya *et al.*, [20].

Table-2: Gender wise distribution in various studies

Study series	Year	Male (%)	Female (%)	Ratio
Lodise *et al.*, [22]	2004	61	38	1.60:1
Viren Javiya *et al.*, [20]	2006	62	37	1.67:1
Rajat Rakesh M *et al.*, [21]	2010	61	39	1.56:1
Present study	2014	65	35	1.85:1

In this study Pseudomonas isolates were more common in males (65%) compared to females (35%) with a male: female ratio of 1.85:1.This is comparable with studies of Viren Javiya *et al.*, [20].The sex wise and age wise distribution of patients diagnosed with infections followed the natural epidemiological pattern [20, 21].

Table-3: Ward wise distribution in various studies

Study series	Year	Surgical ward	Medical ward	ICU
Shampa Anupurba *et al.*, [24]	2005	42.5	30.1	26.9
Angadi KM *et al.*, [23]	2010	52.8	32.7	14.4

Present study	2014	48	41	11

Ward wise distribution of patient in our study is in accordance with study of Angadi KM *et al*., [23]. Prevalence of infection was higher in surgical ward as maximum isolates were isolated from pus/swab samples. This might be due to the prolonged stay in hospital following an operation resulting in colonization and subsequent infection [24]. Use of indwelling medical devices is common in these areas, which play an important role in the spread of infective agents and also the injudicious use of antibiotics which confers resistance to higher drugs [25].

Table-4: Distribution of P.aeruginosa in various clinical samples

Study Series	Year	Pus	Miscellaneous	Blood	Urine	Sputum
Dr.Wankhede *et al*., [26]	2008	44.1	22.9	04	25.2	04
Deepak Arora *et al*., [27]	2010	28	12	14	36	10
Rakesh R M *et al*., [21]	2012	71	-	-	16	12
Present study	2014	41	20	14	15	07

In our study, maximum number of MDR producers were from pus/swab samples, which is comparable with studies done by Dr. Wankhede *et al*., [26] and Rakesh R M *et al*., [21].but MDR producing Pseudomonas aeruginosa were commonly isolated from urine (36%) and blood (14%) in study of Deepak Arora *et al*., [27] . The high numbers of MDR producers in the present study are isolated from pus and body fluids reveals that such organisms might have been acquired by the patients from the hospital environment. It is also evident that there is a distinct difference in the sensitivity pattern of isolates of P. aeruginosa from specimen to specimen which also an identical finding with other studies [28].

Table-5: Antibiotic sensitivity pattern of P.aeruginosa in various isolates

Study series	Year	Antibiotics						
		CF	PC	CAZ	RC	GM	AK	IMP
Ibukun *et al*., [2]	2007	-	30.9	79.4	40.2	42.3	78.4	95.9
Gad G *et al*., [29]	2007	10	-	-	41	26	85	-
Prashant D *et al*., [9]	2011	49.2	58.7	47	18.8	61.9	63.5	31
Rakesh R *et al*., [21]	2012	-	-	57	51	37	-	86
Present Study	2014	72	28	89	57	45	40	32

On comparing the sensitivity patterns observed in present study and previous studies, it shows that clinical isolates of Pseudomonas aeruginosa are becoming resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics. Multi drug resistant P. aeruginosa is an emerging problem. Cefotaxime and Ceftazidime are the commonest 3rd generation antibiotics in hospital protocols. Resistance to 3rd generation cephalosporins and aminoglycosides are significant in our study. This is similar to the study done by Ibukun *et al*., [2], Prashant d *et al*., [9], Rakesh R *et al*., [21] resistance to cephalosporins was high due to the production of extended spectrum β-lactamses (ESBLs) by the bacteria involved. In our study, resistance to amikacin was still lower than to gentamicin and this correlates with the study done by Poole *et al*., [30].in contrast in studies of Ibukun *et al*., [2], Prashant D *et al*., [9], Rakesh R *et al*., [21] , anti pseudomonal effect of amikacin is higher then gentamycin . So, among the aminoglcosides, amikacin has the highest sensitivity .So, Amikacin seems to be a promising therapy for pseudomonas infection. Hence, its use should be restricted to severe nosocomial infections. In present study, resistance to imipenem was 32% similar to study of Prashant *et al*., [9] .but in studies of Ibukun *et al*., [2], Rakesh R *et al*., [21] ,Imipenem showed very high resistnace 95.9% and 86%. This may be due to reduced levels of drug accumulation or increased expression of pump efflux. The production of metallo-β-lactamase (carbapenemase) by the organism can also be a possible factor. There is distinct difference in the sensivitity pattern of isolates of pseudomonas among various studies. This might be due to the environmental condition of this particular region, genetic background of organism or frequent use of antibiotics among patients. This indicates that the sensitivity pattern changes from hospital to hospital and population to population. Thus, as emphasized by various international authorities that every hospital should have its individual antibiotic sensitivity pattern since the standard antibiotic sensitivity pattern may not hold true for every area. This study shows that nowadays the common antimicrobial agents are losing their efficacy against pathogens like P .aeruginosa. This has been possibly resulted from indiscriminate use of antibiotic, lack of awareness, patient non compliance and unhygienic conditions. The irrational and inappropriate use of antibiotics is responsible for the development of

resistance of Pseudomonas species to antibiotic monotherapy.

These results warn us for implementation of infection control measures to limit intra-institutional spread of these organisms [31]. It is the need of the hour that antibiotic policies should be formulated and rationale use of drugs should be implemented to resist and overcome this emerging problem. Every effort should be made to prevent spread of resistant organisms [32].

CONCLUSION

Therefore early detection and prompt instillation of infection control measures is important to prevent spread of MDR to other gram negative rods. Additionally it is also important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics. There should be surveillance programs for the detection of MDR organisms in every locality. Infection control programs need to be implemented with quality control in every hospital.

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