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ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *PSEUDOMONAS AERUGINOSA* ISOLATES FROM BURN WOUNDS

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ABSTRACT
In present study fifty pus samples of burn wound infections were studied for isolation and identification of causative agents. Out of fifty, twenty pus samples showed the presence of *Pseudomonas aeruginosa* organisms. These 20 isolates were studied for antimicrobial susceptibility against antimicrobial agents like Amoxicillin, Cloxacillin, Erythromycin, Tetracycline, Penicillin, Cotrimoxazole, PenicillinV, Cephalexin, Ciprofloxacin, Ofloxacin, Sparofloxacin, Gatifloxacin, Aztreonam, Azithromycin, Vancomycin, Doxycycline etc. All isolates showed high level of resistance to one or more of above antimicrobials.

INTRODUCTION

Infection in the burn patient is a leading cause of morbidity and mortality and remains one of the most challenging concern of the burn team. *Pseudomonas aeruginosa* is regarded as one of the most frequent causes of nosocomial infections (Shobha Ram *et al.,* 2000, Rastegar *et al.,* 2005). This organism usually appears as an opportunistic pathogen which can invade patient with burn wounds. Moreover the organism has an unusual resistance to most routinely used antimicrobials like Amoxicillin, Cloxacillin, Erythromycin, Tetracycline, Penicillin,Cotrimoxazole, PenicillinV, Cephalexin, Ciprofloxacin, Ofloxacin, Sparofloxacin, Gatifloxacin, Aztreonam, Azithromycin, Vancomycin, Doxycycline etc (Po-Rem Hsueh *et al.,*1998). Development of resistance to antimicrobial drugs is caused by a variety of mechanisms such as changes at the site of drug penetration, alteration of drug target sites or production of drug inactivating enzymes. Resistance to variety of antimicrobial drugs is emerging in bacterial pathogens throughout the world. When infection caused by *Pseudomonas spp.* occurs in burn patients the treatment becomes very difficult and mortality rate among the infected patient is likely to reach up to 40 to 50 %. The treatment of these infections is frequently complicated by antimicrobial drug resistance, the problem that has been increasing over the time (Richard *et al.,* 1994). The emergence of multi drug resistant strains in burn unit particularly in economically underdeveloped and developing countries like India is an increasing infection control problem. Since the accuracy of the antimicrobial susceptibility data is associated with performance standard of the test, strict adherence to the standard procedure is essential. The Kirby-Bauer disc diffusion susceptibility test, performed in accordance to NCCLS (National Committee for Clinical Laboratory Standards, 1997) method gives reliable results and hence predicts clinical efficacy of the antimicrobials tested. In this study isolation, identification of pathogen and their antimicrobial susceptibility patterns were studied.

MATERIALS AND METHODS

The pus samples from the burn wounds were collected (Sydney and Ellion Baron, 1986 and Basu *et al.,* 1986) aseptically with sterile cotton swabs from the burn patients admitted in Dr. Jagadale Mama Hospital, Barshi. (Dist. Solapur M. S., India). In each case the swab was taken after the clinical judgment of infection. Infection and septicemia were suspected when a patient showed a sign of disorientation, hyperpyrexia or hypothermia, circulatory embarrassment, petechial haemorrhages, black and dark discoloration in previously clean appearing wounds. The swabs were taken from the site where the actual pus was seen. Cotton swabs of wound exudates were inoculated in sterile peptone water and incubated at 37ºC for 24 hr. After incubation loopful of these samples were streaked on sterile nutrient agar plates and incubated at 37ºC for 24 hr. After incubation the suspected isolated colonies were identified unto species level by convential methods. The antimicrobial susceptibility testing was done for all

Pseudomonas aeruginosa isolates using Kirby-Bauer method on Muller and Hinton agar with disc diffusion technique (Bary and Thorns, 1985). The antimicrobial agents used in polydisc (Hi Media Mumbai) were Amoxicillin, Cloxacillin, Erythromycin, Tetracycline, Penicillin, Cotrimoxazole, PenicillinV, Cephalexin, CiprofloxacinOfloxacin, Sparofloxacin, Gatifloxacin, Aztreonam, Azithromycin Vancomycin, Doxycycline etc. The isolates were inoculated in sterile peptone water and incubated at 37ºC for 18 hr. The sterile plates of Muller and Hinton agar were used for susceptibility testing. The 18 hr. old broth cultures were spread with sterile glass spreaders on sterile plates of Muller and Hinton agar and the polydiscs of antimicrobial agents were placed on the plates. The amount of antimicrobial agents in each disc is shown in Table 4. The plates were kept at 4 to 8ºC temperature for 30 min and then incubated at 37ºC for 18 to 24 hr. After incubation the diameters of the zones of growth inhibition created around the discs were determined. Each zone of inhibition to the nearest millimeter was measured and compared with standard zone size interpretative chart (Table 4) to find out whether the organism is sensitive, resistant or possesses intermediate sensitivity to that particular antimicrobial agent. The degree of susceptibility or resistance of the isolates to each antimicrobial agent was classified based on guidelines of antimicrobial susceptibility testing by Hi Media Lab Mumbai (Table 4)

RESULTS AND DISCUSSION

The isolates from burn wound pus exudates were identified as *Pseudomonas aeruginosa* from the results of cultural, morphological and biochemical study (Table 1). L-Lactose, G-Glucose, M-Manitol, Ox- Oxidase, Ur- Urease, TSI S- Triple Sugar Iron Agar Slant, TSI B- Triple Sugar Iron Agar Butt, GL-Gelatin Liquefaction tests Negative test and +- positive test. The Gram staining showed that all the isolates were Gram negative, rod shaped bacteria. They were non sporulating and motile.The colonies were small around 2 to 3 mm in diameter, flat and bluish green in color. The biochemical characters are shown in Table 1 All the isolates were found to ferment glucose and produce acid but not mannitol and lactose. The isolates were oxidase positive and urease negative. All showed TSI test positive on slants but negative in butt. Gelatin was liquefied by all isolates. As per results (Table 2, 3 and 4) it is evident that susceptibility of *Pseudomonas aeruginosa* strains to different antimicrobial agents varied depending upon isolate. Majority of isolates were resistant to antimicrobial agents tested. From above results it seems that all the Pseudomonas strains are resistant to Amoxicillin, Penicillin and Aztreonam. Ninty percent strains were resistant to Sparofloxacin while resistance shown to Erythromycin and Tetracycline is eighty percent. All strains showed 40 t0 70 % resistance to remaining antimicrobial agents. Similar investigation was done by Shalit *et al.,* (1992) and reported that susceptibility of clinical isolates of *Pseudomonas aeruginosa* to Fluroquinolones. He found that 22% isolates were resistant to Ciprofloxacin, 50% to Ofloxacin and 69% to Pefloxacin. Khond *et al*. (2006) reported the sensitivity of *Pseudomonas aeruginosa* to Fluroquinolones. Amongest Fluroquinolones. Ciprofloxacin and Sparofloxacin were found to be highly effective (100%) while in case of Norfloxacin and Ofloxacin was 99%. Only in case of Lomefloxacin 40% of the strains of *Pseudomonas aeruginosa* were found to be sensitive while other were intermediate.The work of Sugandhi and Shivananda (1983) shows that *Pseudomonas aeruginosa* isolates were resistant to commonly used antimicrobials like Carbenicillin and Gentamycin. George *et al*. (1990) reported a gradual decrease in susceptibility to Ciprofloxacin from 98.6% in 1985 and 86% during 1989. Ojha and Deodhar (1997) noted that the gradual rise in resistance of *Pseudomonas aeruginosa* during their study period from 1994-96. Alexander (1971) also showed that burn injury infections can be controlled by using different antibiotics. In present study it was observed that all the strains were resistant to Amikacin

Table1: Morphological and Biochemical studies of isolates

Sr.No.	*Ps.aeruginosa*Isolate No.	Gram Nature	Motility	L	G	M	Ox	Ur	TSIS	TSIB	GL
1	PS 1	G-ve	motile	-	+	-	+	-	+	-	+
2	PS 2	G-ve	motile	-	+	-	+	-	+	-	+
3	PS 3	G-ve	motile	-	+	-	+	-	+	-	+
4	PS 4	G-ve	motile	-	+	-	+	-	+	-	+
5	PS 5	G-ve	motile	-	+	-	+	-	+	-	+
6	PS 6	G-ve	motile	-	+	-	+	-	+	-	+
7	PS 7	G-ve	motile	-	+	-	+	-	+	-	+
8	PS 8	G-ve	motile	-	+	-	+	-	+	-	+
9	PS 9	G-ve	motile	-	+	-	+	-	+	-	+
10	PS 10	G-ve	motile	-	+	-	+	-	+	-	+
11	PS 11	G-ve	motile	-	+	-	+	-	+	-	+
12	PS 12	G-ve	motile	-	+	-	+	-	+	-	+
13	PS 13	G-ve	motile	-	+	-	+	-	+	-	+
14	PS 14	G-ve	motile	-	+	-	+	-	+	-	+
15	PS 15	G-ve	motile	-	+	-	+	-	+	-	+
16	PS 16	G-ve	motile	-	+	-	+	-	+	-	+
17	PS 17	G-ve	motile	-	+	-	+	-	+	-	+
18	PS 18	G-ve	motile	-	+	-	+	-	+	-	+
19	PS 19	G-ve	motile	-	+	-	+	-	+	-	+
20	PS 20	G-ve	motile	-	+	-	+	-	+	-	+

L-Lactose, G-Glucose, M-Manitol, Ox- Oxidase, Ur- Urease, TSI S- Triple Sugar Iron Agar Slant, TSI B- Triple Sugar Iron Agar Butt, GL- Gelatin Liquefaction tests
-: Negative test and +- positive test

Table 2: The diameters of growth inhibitory zones (in mm) of isolates

Sr.No	Isolate No	Am	Cx	E	T	P	Co	Pv	Cp	Cf	Of	Sc	Gf	Ao	At	Va	Do
1	PS 1	-	-	10	8	-	-	-	-	-	12	-	18	-	20	-	-
2	PS 2	-	-	10	-	-	18	-	-	24	20	18	15	-	10	-	-
3	PS 3	-	-	-	-	-	15	-	-	20	18	-	24	-	-	-	-
4	PS 4	-	-	-	-	-	-	-	-	20	18	10	14	-	-	-	-
5	PS 5	-	-	-	-	-	10	20	20	22	14	-	-	10	-	10	15
6	PS 6	-	-	-	15	-	20	-	-	24	24	20	24	10	18	10	-
7	PS 7	-	-	-	10	-	18	-	-	-	-	-	20	-	-	-	18
8	PS 8	-	-	10	12	-	-	-	-	15	15	10	15	-	26	-	-
9	PS 9	-	-	16	10	-	-	-	-	14	20	-	20	-	-	-	16
10	PS 10	-	-	-	-	-	-	-	-	16	14	-	18	-	20	-	-
11	PS 11	-	-	-	-	-	12	-	-	20	20	18	20	-	26	-	14
12	PS 12	-	-	10	15	-	-	-	-	20	-	14	16	-	-	-	-
13	PS 13	-	10	-	18	-	8	-	-	-	12	-	-	-	20	-	-
14	PS 14	-	-	16	-	-	-	-	-	-	-	-	-	-	18	-	-
15	PS 15	-	-	-	-	-	20	-	-	-	-	-	10	-	20	-	-
16	PS 16	10	5	20	10	-	22	14	-	12	14	-	10	-	20	22	14
17	PS 17	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-
18	PS 18	-	-	-	-	-	-	-	-	20	-	10	10	-	20	-	-
19	PS 19	-	-	-	14	-	26	-	-	26	20	10	22	-	10	-	-
20	PS 20	-	-	10	18	-	-	-	-	20	12	-	18	-	-	-	-

-: No inhibition Zone

Table 3: Results of antibacterial susceptibility testing of isolates of Pseudomonas *aeruginosa*

Sr.No	Isolate	Antibiotic/Drug type															
		Am	Cx	E	T	P	Co	Pv	Cp	Cf	Of	Sc	Gf	Ao	At	Va	Do
1	PS 1	R	-	R	R	R	R	-	-	R	R	R	S	R	S	-	S
2	PS 2	R	-	R	R	R	S	-	-	S	S	I	I	R	R	-	R
3	PS 3	R	-	R	R	R	I	-	-	I	S	R	S	R	R	-	R
4	PS 4	R	-	R	R	R	R	-	-	I	S	R	R	R	R	-	R
5	PS 5	R	-	R	R	R	R	-	-	S	R	R	R	R	R	-	I
6	PS 6	R	-	R	I	R	S	-	-	S	S	S	S	R	S	-	R
7	PS 7	R	-	R	R	R	S	-	-	R	R	R	S	R	R	-	S
8	PS 8	R	-	R	R	R	R	-	-	R	I	R	I	R	S	-	R
9	PS 9	R	-	I	R	R	R	-	-	R	S	R	S	R	R	-	S
10	PS 10	R	-	R	R	R	R	-	-	I	R	R	S	R	S	-	R
11	PS 11	R	-	R	R	R	I	-	-	I	S	I	S	R	S	-	I
12	PS 12	R	-	I	I	R	R	-	-	I	R	R	I	R	R	-	R
13	PS 13	R	-	R	I	R	R	-	-	R	R	R	R	R	S	-	R
14	PS 14	R	-	I	R	R	R	-	-	R	R	R	R	R	S	-	R
15	PS 15	R	-	R	R	R	S	-	-	R	R	R	R	R	S	-	R
16	PS 16	R	-	I	R	R	S	-	-	R	R	R	R	R	S	-	I
17	PS 17	R	-	R	R	R	R	-	-	R	R	R	R	R	R	-	R
18	PS 18	R	-	R	R	R	R	-	-	I	R	R	R	R	S	-	R
19	PS 19	R	-	R	R	R	S	-	-	S	S	R	S	R	R	-	R
20	PS 20	R	-	R	I	R	R	-	-	I	R	R	S	R	R	-	R

Penicillin and Aztreonam. Out of all 90% strains were resistant to Sparofloxacin and 80% to Erythromycin and Tetracycline. The resistance shown by *Pseudomonas aeruginosa* strains to Doxycycline was 70%, Cotrimoxazole and Ofloxacin were 60%, Azithromycin was 50% and for Cifrofloxacin and Gatifloxacin were 40%. If we compare present results to the previous reports it is evident that the present isolates showed fairly high multiple drug resistance to different antimicrobials used and the selective pressure from the use of antimicrobial agents can be major cause of the emergence of resistant strains. The subinhibitory antibiotic concentrations in the burn wounds due to the administration of an inappropriate doses of B- lactam antibiotics or the regular administration of an aminoglycoside in combination with B –lactam provides optimal conditions for the selection and the persistence of the multidrug resistant *Pseudomonas aeruginosa* strains and their subsequent local invasion and haematogenous dessimination in burn patients . The concomitant use of multiple antimicrobial agents including those with good activity against *Pseudomonas aeruginosa* might have contributed to the emergence of the epidemic strains in patients .This also may have contributed to the long term colonization and the subsequent spread of the epidemic multidrug resistant strains in burn patients.The results of this study can be used to evaluate the effects of the changes in burn treatment and antimicrobial resistance development in relation to antibiotic usage. Despite advances

Table 4: Percentage (%) susceptibility of *Pseudomonas aeruginosa* strains to various antimicrobial agents (20 Strains)

Sr.No	Name of Antimicrobial agent	Symbol	Disc content(mcg)	% Resistance and Susceptibility patterns		
				Resistant strains of *Pseudomonas*	Intermediate strains of *Pseudomonas*	Sensitive strains of *Pseudomonas*
1	Amoxicillin	Am	10	100	0	0
2	Cloxacillin	Cx	5	-	-	-
3	Erythromycin	E	15	80	20	-
4	Tetracyclin	T	10	80	20	0
5	Penicillin	P	2 units	100	0	0
6	Cotrimoxazol	Co	25	60	10	30
7	Penicillin-V	Pv	3	-	-	-
8	Cephalexin	Cp	30	-	-	-
9	Ciprofloxacin	Cf	5	40	20	20
10	Ofloxacin	Of	5	60	5	35
11	Sparofloxacin	Sc	5	90	5	5
12	Gatifloxacin	Gf	5	40	20	40
13	Aztreonam	Ao	30	100	0	0
14	Azithromycin	At	15	50	10	40
15	Vancomycin	Va	30	-	-	-
16	Doxycycline	Do	30	70	15	15

in sanitation facilities and the introduction of wide variety of antimicrobial agents having antipseudominal activities, life threatening infections are caused by *Pseudomonas aeruginosa*. Careful surveillance of infection, good isolation techniques and routine procedure and a restrictive antibiotic policy can keep antimicrobial resistance rates low in burn patients. It is also very important to pay more attention to take care of patients who are admitted in burn units. So it is therefore necessary to control the flow of visitors in the burn units as well as strictly enforcing hand washing before and after handling a patients so as to curtail the risk of cross infections and spread of multidrug resistant bacteria such as *Pseudomonas aeruginosa* to others. Thus antimicrobial sensitivity and resistance patterns obtained for *Pseudomonas aeruginosa* isolates in present study must be taken as an alarm in the hospitals from rural places like Barshi.

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