Available Online at http://www.resealert.com

ISSN: 2319-6505

International Journal of Current Advanced Research
Vol1., Issue, 2, pp.26 - 31, October, 2012

International Journal of Current Advanced Research

RESEARCH ARTICLE

Studies on Isolation and Characterization of Some Wound Infection Causing Bacteria

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ARTICLE INFO

Article History:

Received 10th August, 2012
Received in revised form 25th, August, 2012
Accepted 15th September, 2012
Published online 30th October, 2012

Key words:

Wound infecting bacteria, Beta lactamase assay, antibiotics and Virulence factors.

ABSTRACT

The antimicrobial resistance pattern of aerobic bacteria isolated from burn patients admitted in plastic surgery and general surgery wards of Kumaramangalam memorial medical hospital Salem in Tamilnadu. Different types of wound samples were collected from 25 patients during the study period. Among the 25 patients, 5 types of bacterial species were isolated by selective culture medium and standard bio chemical test. Each wound samples showed one more isolates . The five isolates included Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were predominant isolates (40%) followed by Klebsiella pneumoniae and Streptococcus mutans (28%). In Escherichia coli Among the 5 different antibiotics 40% resistance showed by ciprofloxacin In Klebsiella pneumoniae Among the 5 antibiotics 28% of isolates resistance to nalidixic acid and ciprofloxacin, norfloxacin, gentamycin and tobramycin showed sensitive and intermediated to isolates. In Staphylococcus mutans , the highest resistance were showed by ampicillin (57.1%) In Pseudomonas aeruginosa , the highest resistance were showed by gentamycin (50%) In Streptococcus aureus, the highest resistance were showed by ampicillin and penicillin (90%). In Cell surface hydrophobicity, among the 39 isolates the highest activity observed from Pseudomonas aeruoginosa(98.98±0.04%). In Protease enzyme production, Totally 23% of isolates produced protease activity. In β lactamase production, Totally 76.9% of isolates produced betalactamase activity. In Slime production, (Biofilm) all bacterial isolates produced slime activity. According to previous studies, bio film was attached to glass tube surface as positive activity.

The Change in the pattern if bacterial resistance in the burn unit is important both for clinical settings and epidemiological purposes.

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INTRODUCTION

Isolation of wound infection causing bacteria

Wound and skin infections represent the invasion of tissues by one or more species of microorganism. This infection triggers the body's immune system, causes inflammation and tissue damage, and slows the healing process. Many infections remain confined to a small area, such as an infected scratch or hair follicle, and usually resolve on their own. Others may persist and, if untreated, increase in severity and spread further and/or deeper into the body.

Skin and wound infections interfere with the healing process and can create additional tissue damage. They can affect anyone, but those with slowed wound healing due to underlying conditions are at greater risk. Bowler (1998).

Pathogenic effects of virulent micro-organisms

Toxin and Super antigen production

When production of toxin, Vigorous the stimulation of immune cells. These toxins tend to cause local necrosis and disrupt the delicate balance of critical mediators such as cytokines and proteases necessary for healing progression (Ovington, 2003).These super antigen also initiates an uncontrolled proliferation of T cells.

Antibiotic resistance of wound isolates

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistant *Enterobacter* (VRE). Most bacteria have multiple routes of resistance to any drug and, once resistant, can rapidly produce vast numbers of resistant progeny (Livermore, 2003).

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics.

MATERIALS AND METHODS

Collection of wound pus samples

A totally 35 pus swabs were obtained from wound sites before the wound was cleaned using an antiseptic solution. The specimen was collected on sterile cotton swab without contaminating them with skin commensals. All samples were collected from in around Namakkal area hospitals and properly labeled indicating the source and age of patients.

Isolation and identification of wound isolates

Culture plates of Eosin Methylene Blue Agar, MacConkey Agar, Nutrient Agar, Citramide agar and Mannitol Salt Agar were used.

Characterization and identification of the isolates was done using the methods of Cowan (1985); Fawole and Oso's (1988) and Cheesbrough (2004).

PRELIMINARY TEST

The samples were subjected to the following tests

Gram staining

- Motility test
- Catalase test
- Oxidase test

BIOCHEMICAL TESTS

The samples were subjected to the following tests

Indole test

- Tryptone broth

Kovac's reagent

Para-dimethyl amino benzaldehyde	:	5.0 g
Butyl alcohol	:	75 ml
Conc. hydrochloric acid	:	25 ml

Methyl red test

Culture was inoculated with Methyl red – Voges proskauer (MR-VP) broth and incubated for 48 – 72 hrs at 37°C. The appearance of a red color on addition of methyl red solution was considered as positive.

Glucose – phosphate broth (MR-VP)
Voges – proskauer test

Culture was inoculated with MR - VP medium and incubated at 37°C for 24-48 hrs. After incubation, 3 ml of Barrit's reagent A and one ml of Barrit's reagent B were added. The tubes were shaken and allowed to stand for 15 minutes and observed for colour change. The development of pink colour was considered as positive.

Barrit's reagent A

5% alpha naphthol	:	5.0 g
Absolute ethanol	:	95 ml

Barrit's reagent B

Potassium hydroxide	:	40 g
Creatine	:	3 g
Distilled Water	:	1000 ml

Test for H_2S production and glucose utilization

Culture was inoculated with Triple sugar iron agar slants and incubated at 37°C for 24 hrs. The change in colour of the medium from red to yellow indicated the production of acid from glucose. A blackening of the medium indicates production of H_2S. Break in the medium show production of gas from glucose.

Urease test

Antibacterial stability test

The standard Kirby Bauer disk diffusion method was used to determine the antimicrobial profile of the wound isolates against 9 antimicrobial agents such as tetracycline, ampicillin, erythromycin, ciprofloxacin and kanamycin.

Characterization of wound bacterial isolates

Assay for beta lactamase production

Beta lactamase production was assayed using the method of Lateef (2004).

Cell surface hydrophobicity

Microbial surface hydrophobicity was assessed with xylene according to Siegfried *et al.,* 1994; Raksha *et al.,* 2003.

Protease enzyme production

Qualitative assay (Kubaran *et al.,* 2010)
Slime activity (Mathur et al., 2006)

RESULT AND DISCUSSION

Isolation and identification of bacteria from wound

Different types of wounds samples were collected from 25 patients during the study period. Among the 25 patients, 5 types of bacterial species were isolated by selective culture medium and standard biochemical test. Each wound samples showed one more isolates. The five isolates included *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were predominant isolates (40%) followed by *Klebsiella pneumoniae* and *Streptococcus.mutans* (28%). The results were tabulated in Table 1.

Streptococcus aureus

The highest resistance were showed by ampicillin and penicillin (90%) second most chlorophenical (70%) followed by gentamycin (60%), tetracycline (40%). The result was tabulated in Table 2, 3 and 4.

Virulence character of wound isolates Cell surface hydrophobicity

Among the 39 isolates the highest activity observed from *Pseudomonas aeruoginosa*(98.98±0.04%). Second most *E.coli* (98.87±0.03) followed by *S.mutans* (95.91±0.07), *K.pneumoniae* (93.27±0.02) and *S.aureus* (87.25±0.02).

Table 1 Prevalence of Bacterial isolates from different wound samples

S.No	Samples	Nature of wound	Sources	Organisms isolated
1.	1	Burn	Leg	*Staph, Kleb*
2.	2	Accident wound	Leg	*Kleb, Pseudo*
3.	3	Trauma	Leg	*Staph,*
4.	4	Burn	Hand	*Proteus mirabilis, Pseudo*
5.	5	Skin infection	Arms	*Staph, Strep, Pseudo*
6.	6	Accident wound	Leg	*Kleb, Pseudo,*
7.	7	Post operation sepsis	Leg	*Staph*
8.	8	Burn	Hand	*Proteus mirabilis, Proteus vulgaris*
9.	9	Abscesses	Hand	*E.coli, Staph. Strep*
10.	10	Burn	Leg	*Staph,Kleb*
11.	11	Burn	Hand	*Kleb*
12.	12	Trauma	Leg	*E.coli, Staph, Strep*
13.	13	Trauma	Leg	*Staph,*
14.	14	Accident wound	Leg	*Staph,*
15.	15	Burn	Hand	*Proteus mirabilis, E.coli,*
16.	16	Burn	Hand	*Proteus mirabilis and vulgaris E.coli*
17.	17	Abscesses	Abdomen	*Proteus mirabilis,*
18.	18	Skin infection	Wrist	*Strep, Pseudo*
19.	19	Accident wound	Arms	*Kleb,Pseudo,Staph*
20.	20	Skin infection	Leg finger	*Staph, Strep, Pseudo*
21.	21	Burn	Hand	*Kleb, Proteus mirabilis,Pseudo*
22.	22	Abscesses	Abdomen	*Nil*
23.	23	Accident wound	Leg	*Pseudo*
24.	24	Skin infection	Wrist	*pseudo, Strep, E.coli*
25.	25	Trauma	Leg	*Strep, Staph*

Effect of antibacterial agents on the wound isolates escherichia coli

Among the 5 different antibiotics 40% resistance showed by ciprofloxacin and second most co-trimoxazole (20%) and other antibiotic showed sensitive or intermediated results.

Klebsiella pneumoniae

Among the 5 antibiotics 28% of isolates resistance to nalidixic acid and ciprofloxacin, norfloxacin, gentamycin and tobramycin showed sensitive and intermediated to isolates.

Staphylococcus mutans

The highest resistance were showed by ampicillin (57.1%) second most penicillin (42.8%), gentamycin (28.5%), tetracycline (14.2%) and chlorophenical showed sensitive to all isolates.

Pseudomonas aeruginosa

The highest resistance were showed by gentamycin (50%) followed by tobramycin (40%), followed by nalidixic acid and norfloxacin (30%), ciprofloxacin (20%).

Protease enzyme production

Totally 23% of isolates produced protease activity. Among them *S.mutans* (57.1%) were highly produced activity second *S.aureus* (30%) followed by *Pseudomonas* (20%). In this study no activity observed from *Kleb* and *E.coli.*

β lactamase production

Totally 76.9% of isolates produced betalactamase activity. The highest Beta lactamase activity was observed from*Klebsiella* (100%) second most Pseudomonas isolates (90%) followed by, *S.mutans* (85.7%) and E.coli (60%) and lowest activity from *S.aureus* (50%).

Slime production (Biofilm)

In this investigation all bacterial isolates produced slime activity. According to previous studies, biofilm was attached to glass tube surface as positive activity. The result was tabulated in Table 5 and 6 and Figure - 1

The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients. The aim of the present study was to obtain information about

Table 2 Effect of antibiotic agents on wound isolates

Sl. No.	Antibiotics	Resistant	Sensitive	Intermediate
		Escherichia coli		
1.	NA	-	60	40
2.	CIP	40	60	-
3.	Co	20	60	-
4.	AMP	-	100	-
5.	NF	-	80	20
		Klebsiella pneumonia		
1.	CIP	-	71.42	28.57
2.	NA	28.57	42.85	28.57
3.	Nx	-	57.14	42.85
4.	GEN	-	100	-
5.	TB	-	100	-
		Staphylococcus mutans		
1.	TE	14.28	71.42	14.28
2.	GEN	28.57	71.42	-
3.	AMP	57.14	42.85	-
4.	P	42.85	42.85	14.28
5.	C	-	85.71	14.28
		Pseudomonas aeruginosa		
1.	CIP	20	50	30
2.	NA	30	50	20
3.	NX	30	50	20
4.	GEN	50	50	-
5.	TB	40	60	-
		Streptococcus aureus		
1.	TE	40	40	20
2.	GEN	60	20	20
3.	AMP	90	-	10
4.	P	90	10	-
5.	C	70	-	30

NA – Nalidixc acid CIP – Ciproflaxin Co – Co-Trimozole Amp– Ampicillin GEN- Gentamycin NF – Norfloxain TB – Tobramycin P – Penicillin, C – Chloramphenicol TE- Tetracycline

the type of isolates, identification and characterization of bacterial wound infections. (Mohammed *et al.*, 2011)

In the present study, the most commonly isolated organisms from burned patients were P. aeruginosa followed by S. aureus, and K. pneumoniae. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents, or the immunosuppressive effects of burn truma. Our results showed that the rate of isolation of gram-negative organism was more than gram-positive, these results are in correlates with the work of Mohammed *et al.*, (2011). The change in the pattern of bacterial resistance in the burn unit is important both for clinical settings and epidemiological purposes.

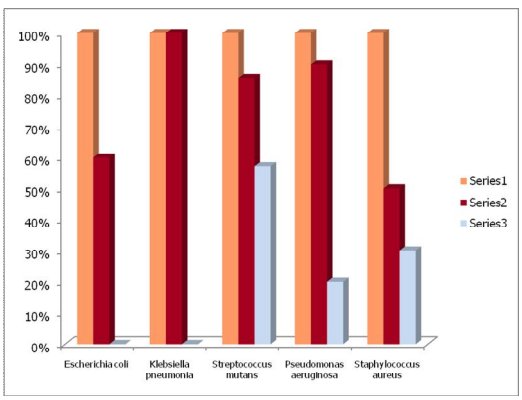

Fig.1

Table 3 Percentage of resistance and sensitivity of all the organisms

Sl.No.	Wound isolates	Resistant	Sensitive	Intermediate
		Escherichia coli		
1	E1	-	80	20
2	E2	20	60	20
3	E4	-	100	-
4	E5	-	80	20
5	E9	20	40	40
		Klebsiella pneumonia		
1	K1	-	100	-
2	K4	-	100	-
3	K5	20	40	40
4	K6	-	80	20
5	K7	-	80	20
6	K8	20	40	40
7	K10	-	80	20
		Streptococcus mutans		
1	ST1	-	100	-
2	ST2	-	80	20
3	ST3	60	-	40
4	ST4	-	100	-
5	ST5	40	60	-
6	ST6	40	60	-
7	ST7	60	40	-
		Pseudomonas aeruginosa		
1	P1	40	20	40
2	P4	80	-	20
3	P5	20	80	-
4	P9	100	-	-
5	P10	20	80	-
6	P11	-	100	-
7	P12	-	100	-
8	P13	-	40	60
9	P14	-	80	20
10	P15	80	20	-
		Staphylococcus aureus		
1	S1	60	20	20
2	S2	60	20	20
3	S3	60	40	-
4	S4	40	40	20
5	S5	60	40	-
6	S9	40	20	40
7	S10	80	20	-
8	S11	60	20	20
9	S12	60	20	20
10	S13	60	20	20

Table 4 Antibiotic susceptibility pattern

Sl.No.	Strain No.	Antibiotics				
		CIP	NA	NF	GEN	TB
		Escherichia coli				
1	E1	I	S	S	S	S
2	E2	S	R	I	S	S
3	E4	S	S	S	S	S
4	E5	S	S	S	S	I
5	E9	I	R	R	S	S
		Klebsiella pneumoniae				
1	K1	S	S	S	S	S
2	K4	S	S	S	S	S
3	K5	I	R	I	S	S
4	K6	S	I	S	S	S
5	K7	S	I	S	S	S
6	K8	I	R	I	S	S
7	K10	S	S	I	S	S

	Streptococcus mutans					
1	ST1	S	S	S	S	S
2	ST2	S	S	S	I	S
3	ST3	I	R	R	R	I
4	ST4	S	S	S	S	S
5	ST5	S	S	R	R	S
6	ST6	R	S	R	S	S
7	ST7	S	R	R	R	S
	Pseudomonas aeruginosa					
1	P1	I	I	S	R	R
2	P4	I	R	R	R	R
3	P5	S	S	S	R	S
4	P9	R	R	R	R	R
5	P10	S	S	S	R	S
6	P11	S	S	S	S	S
7	P12	S	S	S	S	S
8	P13	I	I	I	S	S
9	P14	S	S	I	S	S
10	P15	R	R	R	S	R
	Staphylococcus aureus					
1	S1	S	R	R	R	I
2	S2	R	I	R	R	S
3	S3	S	R	R	R	S
4	S4	S	R	I	R	S
5	S5	S	R	R	R	S
6	S9	I	R	R	S	I
7	S10	R	R	R	R	S
8	S11	R	I	R	R	S
9	S12	R	S	R	R	I
10	S13	I	S	R	R	S

.

Table 6 Virulence factors for slime production

S.no	Wound Isolate	Slime Production	B lactamase production	Protease production
1.	*Escherichia coli*	100%	60%	-
2.	*Klebsiella pneumonia*	100%	100%	-
3.	*Streptococcus mutans*	100%	85.71%	57.14%
4.	*Pseudomonas aeruginosa*	100%	90%	20%
5.	*Staphylococcus aureus*	100%	50%	30%

Table 5 Virulence factors for slime production

S.no	Strain No.	Slime Production	Protease production	B lactamase production
1.	E1	+	-	-
2.	E2	+	-	+
3.	E4	+	-	+
4.	E5	+	-	-
5.	E9	+	-	+
6.	K1	+	-	+
7.	K4	+	-	+
8.	K5	+	-	+
9.	K6	+	-	+
10.	K7	+	-	+
11.	K8	+	-	+
12.	K10	+	-	+
13.	ST1	+	17	+
14.	ST2	+	-	+
15.	ST3	+	-	+
16.	ST4	+	16	+
17.	ST5	+	18	+
18.	ST6	+	18	+
19.	ST7	+	-	+
20.	P1	+	-	-
21.	P4	+	-	+
22.	P5	+	-	+
23.	P9	+	-	+
24.	P10	+	-	+
25.	P11	+	11	+
26.	P12	+	14	+
27.	P13	+	-	+
28.	P14	+	-	+
29.	P15	+	-	+
30.	S1	+	-	+
31.	S2	+	10	+
32.	S3	+	-	-
33.	S4	+	-	+
34.	S5	+	-	+
35.	S9	+	15	+
36.	S10	+	14	-
37.	S11	+	-	-
38.	S12	+	-	-
39.	S13	+	-	-

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