

RESEARCH ARTICLE

Incidence and screening of wound infection causing microorganisms

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Abstract

Different types of wounds samples were collected from 19 patients during March-April 2011 in Namakkal, Tamil Nadu, India. Four types of bacterial species were isolated and identified by selective culture medium and standard biochemical tests from the collected wound samples. Each wound samples showed one or more bacterial isolate, totally 78.9% of samples exhibited 24 isolates. Among them, *Staphylococcus aureus* (54.1%) was the predominant isolate, second most was *Klebsiella pneumoniae* (20.8%) followed by *Pseudomonas aeruginosa* (16.6%) and the lowest percentage was recorded by *Escherichia coli* (8.3%). Highest occurrence was observed in burn wound samples (44%) and second most is the accident and post-operative wound samples (25%) followed by skin wound samples (24%).

Keywords: Wounds samples, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*.

Introduction

Wound infection causes morbidity and mortality in the patients hospitalized for the treatment of burn (Mc Manus *et al.*, 1994). Burn injuries contribute 75% of mortality among the wound infected individuals in the developing countries (Donati *et al.*, 1993). Burn wound facilitate a favourable lodgement for the existence and multiplication of bacteria (Agnihotri *et al.*, 2004). Patients with diminished immunity are highly susceptible and at increased risk of developing a wound infection (Heinzelmann *et al.*, 2002).

There are several factors including age, obesity, malnutrition, endocrine and metabolic disorders influencing development of wound infection. Virulence, quantity and antibiotic resistance of bacteria are also leads to contamination that results in wound infection (Baquero, 1997). Bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Bacteroides fragilis*, *Peptostreptococcus* and *Propionibacterium* spp. were mostly isolated from burn wounds (Lawrence and Lilly, 1972; Riaz and Babar, 1996; Church *et al.*, 2006). Microorganisms that causes infections present in the surfaces of wound are similar to blood (Siddique, 1998). In view of the above facts, this study was aimed to isolate bacterial species from collected wound samples and identifying them by selective culture medium and standard biochemical tests.

Materials and methods

Sample population: Patients included in this study were residents in Namakkal, TN. Male and female were at the age group of 15-45 years. The study was carried out during March-April 2011.

Collection of wound pus samples: A total of 19 pus swabs were obtained from wound sites before the wound was cleaned using 70% alcohol. The specimen was collected on sterile cotton swab without contaminating them with skin commensals. Different types of wound samples were collected namely accident wound, post operation sepsis, skin infection and burn wound. All samples were collected from hospitals in Namakkal and properly labeled indicating the source and age of patients. The samples were transported soon to the laboratory after being obtained. In the laboratory, the specimens were registered and swabs were cultured on nutrient broth and incubated at 37°C for 24 h. Ethical clearance for this part of the study was obtained (IEC No. DM/2011/101/26).

Isolation and identification of wound bacterial isolates: Culture plates of Eosin methylene blue agar, MacConkey agar, Nutrient agar, Citramide agar and Mannitol salt agar (Hi Media, India) were used. The swab sticks used for the collection of the samples were streaked directly on the labeled agar plates and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 h. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Biochemical tests were performed to identify the isolates. Biochemical tests applied were standard catalase test, citrate utilization, coagulase, oxidase, methyl red, Voges-Proskauer, Indole production, motility, carbohydrate fermentation test using glucose, sucrose, maltose and lactose. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985), Cheesbrough (2004), Mathur *et al.* (2006) and Senthilkumar *et al.* (2012).



Table 1. Prevalence of bacterial isolates from wound samples.

Sample	Nature of wound	Source	Age/sex	Organisms isolated
S1	Burn	Leg	18/M	<i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i>
S2	Accident wound	Leg	20/M	<i>Pseudomonas aeruginosa</i> , <i>S. aureus</i> , <i>K. pneumoniae</i>
S3	Skin infection	Leg	15/M	<i>S. aureus</i>
S4	Burn	Hand	28/F	-
S5	Skin infection	Arms	21/F	<i>P. aeruginosa</i> , <i>S. aureus</i>
S6	Accident wound	Leg	20/M	-
S7	Post operation sepsis	Leg	22/M	<i>S. aureus</i>
S8	Burn	Hand	35/F	<i>P. aeruginosa</i>
S9	Skin infection	Hand	38/F	-
S10	Burn	Leg	28/M	<i>S. aureus</i> , <i>K. pneumoniae</i>
S11	Burn	Hand	38/F	<i>Escherichia coli</i> , <i>S. aureus</i> , <i>K. pneumoniae</i>
S12	Post operation sepsis	Leg	45/M	<i>S. aureus</i>
S13	Skin infection	Leg	15/F	<i>S. aureus</i>
S14	Accident wound	Leg	17/M	<i>S. aureus</i>
S15	Burn	Hand	18/F	<i>E. coli</i> , <i>P. aeruginosa</i>
S16	Burn	Leg	18/M	<i>S. aureus</i> , <i>K. Pneumoniae</i>
S17	Skin infection	Stomach	19/M	<i>S. aureus</i>
S18	Skin infection	Neck	38/F	<i>S. aureus</i>
S19	Accident wound	Hand	26/M	-

Table 2. Biochemical test results for bacterial isolates.

Biochemical tests	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Indole test	+	-	-	-
Methyl red test	+	+	-	-
Voges-prokauer test	-	+	-	+
Citrate utilization test	-	-	-	+
Triple sugar ion test	A/A.G(+)	A/A.G(-)	AK/AK.G(-)	A/A.G(+)
Urease	-	-	-	+
Catalase	+S	-	+	+
Glucose	+	+	-	+
Sucrose	+	+	-	+
Maltose	+	+	-	+
Lactose	+	+	-	+

Table 3. Cultural characteristics of bacterial isolates on selective media.

Isolates and media	Cultural characteristics
<i>E. coli</i> in Eosin methylene blue agar	Metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment.
<i>S. aureus</i> in Mannitol salt agar	Colonies that fermented mannitol and appeared golden yellow.
<i>P. aeruginosa</i> in Nutrient agar	Green color pigment.
<i>K. pneumoniae</i> in MacConkey agar	Organisms that ferment lactose and produce the pink colour colony.

Table 4. Percentage of bacterial isolates from various wounds.

Samples	Bacterial isolates (%)				Isolates (%)
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	
Burn	33	57	28	57	43.7
Accident wound	0	50	25	25	25
Skin infection	0	83	16	0	24
Post-operative wound	0	100	0	0	25

Results and discussion

A total of 19 patients with different types of wounds samples were collected during the study period. Four types of bacterial species were isolated and identified by selective culture medium and standard biochemical tests. Each wound samples showed one or more bacterial isolate, totally 78.9% of samples exhibited 24 isolates. Among them, *Staphylococcus aureus* (54.1%) was the predominant isolate, second most was *Klebsiella pneumoniae* (20.8%) followed by *Pseudomonas aeruginosa* (16.6%) and the lowest percentage was recorded by *Escherichia coli* (8.3%) (Table 1). Bacterial isolates were identified using biochemical tests and cultural characteristics (Table 2 and 3).

Gram positive cocci were associated more conspicuously with wound infections in hospitalized patients than in the out patients. There was almost equal prevalence of gram positive and gram negative bacteria in hospitalized patients. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were commonly found in wound infections. Moreover, enterococcal infections are reported very less frequently (Anbumani *et al.*, 2006). Similarly, this study also revealed the prevalence of *S. aureus* other than enterobacteriaceae bacteria such as *K. pneumoniae*, *P. aeruginosa* and *E. coli* in different wound infections. Polymicrobial etiology among *S. aureus* and *P. aeruginosa* occurred frequently in wound infections (Anbumani *et al.*, 2006). This study also revealed poly microbial etiology among *S. aureus* and *K. pneumoniae*.

In this study, highest occurrence were observed in burn wound samples (44%) and second most is the accident and post-operative wound samples (25%) followed by skin wound samples (24%). *Staphylococcus aureus* was the predominant bacterial strain in all samples followed by *P. aeruginosa* (Table 4). Bacterial isolates screened from accident wound, skin infection, burn, post-operation sepsis included *S. aureus* (54.1%), *K. pneumoniae* (20.8%), *P. aeruginosa* (16.6%) and *E. coli* (8.3%). Similarly, various bacterial isolates from various wound infections were reported by Lawrence and Lilly (1972), Riaz and Babar (1996), Church *et al.* (2006) and Muhammad *et al.* (2012).

Conclusion

This study revealed the presence of wound infection causing bacteria, those are capable of causing various human illness. The bacterial isolates screened in various wound infections includes Burn (43.7%), accident wound (25%), post operative wound (25%) and skin infections (24%). The bacterial isolates of different wound infections were *S. aureus* (54.1%), *K. pneumoniae* (20.8%), *P. aeruginosa* (16.6%) and *E. coli* (8.3%). Further, it is necessary to treat wound infected patients with appropriate antibiotics after sensitivity testing. Health education programme and hygienic practices should be adopted in order to avoid community acquired spreading of wound infections causing bacteria.

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