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Research Article

ISOLATION AND SCREENING OF PATHOGENIC BACTERIA FROM WOUND INFECTIONS

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ABSTRACT

Purulent materials from different wound infections were collected aseptically with the aid of sterile swab sticks from 25 patients of Namakkal district. Samples were properly labeled indicating the source, sex, age of patients. Seven 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 43 isolates. Among them, *Staphylococcus aureus* (37.2%) was the predominant isolate, second most was *Pseudomonas aeruginosa* (18.6%) followed by *Streptococcus pyogenes* (13.9%) and the lowest percentage was recorded by *Proteus vulgaris* (2.3%). Highest occurrence was observed in skin wound samples (44%) and second most is the burn wound samples (28%) followed by accident wound (16%).

Keywords: Wounds samples, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Streptococcus pyogenes.*

INTRODUCTION

Wounds are inescapable events of life, which arise due to physical injury, chemical injury and microbial infections. Healing of wounds usually takes place in a direction away from its normal course and under healing, over healing or no healing of wounds is common. Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing (Bennet, 1988).

A wound occurs when the integrity of any tissue is compromised (eg. skin breaks, muscle tears, burns or a bone fracture). A wound may be caused as a result of a fall, a surgical procedure, an infectious disease or an underlying pathological condition. Types and causes of wounds are wide ranging, and healthcare professionals have several different ways of classifying them. They may be chronic, such as the skin ulcers caused by diabetes mellitus, or acute, such as a gunshot wound or animal bite. The knowledge of the physiology of the normal wound healing trajectory through the phases of hemostasis, inflammation, granulation and maturation provides a framework for an understanding of the basic principles of wound healing (Grinnell, 1984).

Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from planned sources are known to increase the healing of different types of wounds. Though some of these drugs have been screened for evaluation of their wound healing activity, the potential of many of the traditional used herbal agents remains unexplored. In few cases active chemical constituents were identified (Biswas and Mukherjee, 2003).

Surgical wound infection is a common post-operative complication and causes significant post-operative morbidity and mortality, prolongs hospital stay, and adds between 10 and 20% to hospital costs. Although the total elimination of wound infection is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both patient comfort and medical resources used. Thermal injury is a serious type of trauma requiring care in specialized units (Deitch, 1990).

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). Bacterial species 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 (n=25) both male and female included in this study were residents of Namakkal, TN.

Collection of wound pus samples

A total of 25 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, abscesses 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 37ºC for 24 h.

Isolation and identification of wound bacterial isolates

Culture plates of Eosin methylene blue agar, MacConkey agar, Nutrient agar, Cetrimide 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).

RESULTS AND DISCUSSION

A total of 25 patients with different types of wounds samples were collected during the study period. Seven 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* (37.2%) was the predominant isolate, second most was *Pseudomonas aeruginosa* (18.6%) followed by *Streptococcus pyogenes* (13.9%) and the lowest percentage was recorded by *Proteus vulgaris* (2.3%). Bacterial isolates were identified using biochemical tests and cultural characteristics (Table 1). 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).

In this study, highest occurrence were observed in skin wound samples (44.2%) and second most is the burn wound samples (27.9%) followed by accident wound samples (16%). *Staphylococcus aureus* was the predominant bacterial strain in all samples followed by *P. aeruginosa* (Table 2). Similarly, various bacterial isolates from various wound infections were reported by Lawrence and Lilly (1972), Riaz and Babar (1996), Church *et al*. (2006), Muhammad *et al*. (2012) and Valarmathi *et al.* (2013)

Table 1: Colony morphology and selective cum differential media

S. No.	Strain number	EMB Agar	XLD Agar	HE Agar	Blood Agar	Baired Parker Agar	Cetrimide Agar
1.	SN 1	Metallic sheen	Yellow	Orange	Non-haemolytic	-	-
2.	SN 2	-	-	-	β-haemolytic	Black	-
3.	SN 3	Dark color	Yellow	Orange	Non-haemolytic,	-	-
4.	SN 4	Pink	Red to yellow	blue-green to blue	Non-haemolytic	Black, No halo	-
5.	SN 5	-	Colourless Some Red	Irregular, green to brown	Non-haemolytic, Rough	-	Bluish green
6.	SN 6	-	-	-	β-haemolytic	Black	-
7.	SN 7	Partial growth colourless	-	Partial growth Yellow	Small gray Non-haemolytic	Black colonies suppressed, no halo	-

Table 2: Isolation of bacteria from different types of wound samples

Name of the samples	Name of the isolates							Total occurrence	Percentage (%)
	SN3	SN7	SN6	SN4	SN1	SN5	SN8		
Burn	4	-	3	4	1	-	-	12	27.9
Accident wound	3	-	1	1	1	-	1	7	16.3
Abscesses	1	1	-	-	-	-	1	3	7
Skin infection	7	5	4	-	2	1	-	19	44.2
Post operative sepsis	1	-	-	-	1	-	-	2	4.7
Total	16	6	8	5	5	1	2		
Percentage (%)	37.2	14	18.6	11.6	11.6	2.3	4.7		

SN1- Escherichia coli, SN2-Staphylococcus sp., *SN3- Klebsiella* sp., *SN4 – Proteus* sp., *SN5- Pseudomonas* sp., *SN6-Strptococcus* sp., *SN7-Enterococcus* sp.

CONCLUSION

This study revealed the presence of wound infection causing bacteria, those are capable of causing various human illness. The bacterial isolate screened in various wound infections includes skin (44.2%), burn wound (27.9%), accident wound (16%), Abscesses (7%) and post operative sepsis (4.7%). The bacterial isolates of predominately wound infections were *S. aureus* (37.2%), compared with others. 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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