Biochemical characterization of bacteria isolated from different wound samples

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Abstract: This study was conducted with an aim to isolate and characterize the bacteria from different wound samples obtained from a local Hospital at Jalandhar, India. The samples were transported to the Microbiology Laboratory, D.A.V University, Jalandhar from the hospital as soon as they were obtained. The microorganisms isolated from these samples were cultured using different media such as Nutrient agar (NA), MacConkey agar (MAC), Mueller Hinton agar (MHA), and Mannitol salt agar (MSA). The spread plate and streaking techniques were used for obtaining pure cultures. The bacteria were morphologically identified by their colour, colony appearance, and gram's staining technique. A total of 13 morphotypes were isolated from five different types of wound samples. The results showed the maximum number of morphotypes obtained were gram-positive. The bacteria were further characterized using different biochemical tests. This study concludes that all types of the wound have different bacterial fauna which may be pathogenic and pose a serious health risk if not taken care of.

Index Terms – Bacteria, Jalandhar, Streaking, Biochemical.

INTRODUCTION

A wound is the loss of a protective layer of skin that can be caused by any accident or operation [1]. The wound can damage the epithelial layer severely, also it can deeply infect the deeper layers of connective tissue. Wound can be of different types including pus, bruises, blisters, burns, and abrasions depending upon the cause. An infection can occur at the site of the wound which could affect the health of an individual. Infection occurs when the virulence factors of microorganisms present in a wound fight with the host immune system [2]. Wound infections may be fatal if untreated [3]. Wound samples have different kinds of bacteria in different types of samples as the study conducted by different scientists all over the world. The majority of bacteria found to be present on burn samples were *S. epidermidis, S. aureus, alpha-hemolytic streptococci, Propionibacterium acnes, Peptostreptococcus spp., Bacteroides spp.* according to the study conducted by Brook and co-workers in 1981 [14]. The study conducted by Brook in 1989 on post-surgical samples the isolates found were *E. coli, Bacteroides spp., Peptostreptococcus spp., Clostridium spp*. [15]. Many wound infections also result from major and minor surgeries [4-6]. The wound infections which are caused by other reasons excluding surgeries can be problematic in poor regions where the health facilities are not good [7-9]. The diagnosis of infection can be difficult in these regions due to the lack of satisfactory equipment [10]. Our present study is focused to isolate and characterize the bacteria present in five different types of wounds.

MATERIAL AND METHODS

Source of the Sample

A total of five sample types were obtained from different patients from a local hospital, Jalandhar, India. Samples were usually from different kinds of wounds such as pus, bruise, post-operative wound, otitis, and blisters. Samples were soon transported to the Microbiology laboratory aseptically within one hour.

Culture of the sample

In the Microbiology laboratory, serial dilution and spread plate methods were used for the isolation of samples which were inoculated on four different media- Nutrient agar (NA), Mac Conkey agar (MAC), Mueller Hinton agar (MHA), and Mannitol salt agar (MSA). These were then incubated at 37^0C for 24 to 48 hours. On the completion of the incubation period, the cultures were examined for bacterial growth. The different morphotypes were identified and the total viable count was calculated by the method of Aneja [11].

Morphological identification and Gram's staining

The primary identification of bacterial isolates was made based on their colony appearance and colour. Gram staining method was used to differentiate bacterial isolates into two groups; Gram-positive and Gram-negative bacteria by detecting peptidoglycan on their cell walls [12].

Biochemical characterization of the isolates

Biochemical tests was performed to identify the unknown bacterial isolates [11]. Biochemical tests applied in this study was catalase test, citrate utilization test, methyl red test, Voges-Proskauer test, Indole test, glucose fermentation test, and lactose fermentation test by using the standard protocols [13].

Statistical analysis

All the tests done were in triplets and their mean were calculated for the main readings.

Results

Isolation and enumeration of bacteria from different wound samples.

The number of morphotypes obtained on different media were examined and their viable count was also counted using a standard protocol, the results of viable count and number of morphotypes are given in Table 1.

Table 1: Viable count and different morphotypes obtained from different wound samples

S.No.	Sample	Sample code	Viable count	Medium	No. of morphotypes
1.	Pus	P-NA	311×10^5 cfu/ml	Nutrient agar	2
2.	Pus	P-MAC	80×10^5 cfu/ml	MacConkey agar	1
3.	Pus	P-MSA	No growth	Mannitol salt agar	0
4.	Pus	P-MH	No growth	Mueller Hinton agar	0
5.	Burn	B-NA	140×10^5 cfu/ml	Nutrient agar	1
6.	Burn	B-MCA	No growth	MacConkey agar	0
7.	Burn	B-MSA	No growth	Mannitol salt agar	0
8.	Burn	B-MH	No growth	Mueller Hinton agar	0
9.	Blister	BS-NA	101×10^5 cfu/ml	Nutrient agar	2
10.	Blister	BS-MAC	65×10^5 cfu/ml	MacConkey agar	2
11.	Blister	BS-MSA	31×10^5 cfu/ml	Mannitol salt agar	1
12.	Blister	BS-MH	No growth	Mueller Hinton agar	0
13.	Otitis	O-NA	No growth	Nutrient agar	0
14.	Otitis	O-MAC	361×10^5 cfu/ml	MacConkey agar	3
15.	Otitis	O-MSA	No growth	Mannitol salt agar	0
16.	Otitis	O-MH	No growth	Mueller Hinton agar	0
17.	Post-surgical	P-SNA	No growth	Nutrient agar	0
18.	Post-surgical	P-SMAC	No growth	MacConkey agar	0
19.	Post-surgical	P-SMSA	No growth	Mannitol salt agar	0
20.	Post-surgical	P-SMH	40×10^5 cfu/ml	Mueller Hinton agar	1

Morphological characterization and Gram staining of bacterial isolates

A total of 13 different bacterial morphotypes were obtained on Nutrient agar, MacConkey agar, Mannitol salt agar, and Mueller Hinton agar media. The bacterial strains were characterized by their colony appearance by observing their size, shape and colour. Gram's staining of bacterial isolates was done which differentiate gram positive bacteria from gram negative bacteria on the basis of thick peptidoglycan layer. The results of these are mentioned in Table 2.

Table 2: Morphological characterization and Gram's staining results

S.No	Sample code	Colony morphology	Gram's reaction
1.	P-NA1	Large, irregular, creamish	+
2.	P-NA2	Small, irregular, creamish	+
3.	B-NA	Circular, greyish white	-
4.	BS-NA1	Coccus, round, elevated	+
5.	BS-NA2	Rod, cream, elevated	-
6.	P-MAC	Circular, red, raised	-
7.	O-MAC1	Red, large, circular, raised	-
8.	O-MAC2	Red, small, circular, raised	-
9.	O-MAC3	Red, circular, raised	+
10.	BS-MAC1	Coccus, red, flat	+
11.	BS-MAC2	Coccus, moderate	+
12.	BS-MSA	Creamish, small, convex	+
13.	P-SMH	Filamentous, rod , gumlike	+

Biochemical characterization of bacterial isolates

All the 13 bacterial isolates which were obtained from different wound samples were characterized biochemically. Series of biochemical tests were conducted such as catalase test, indole test, methyl red test, v-p test, citrate utilization test, glucose fermentation test and lactose fermentation test. The results are shown in the Table 3.

Table 3: Biochemical characterization of bacterial isolates

Bacterial Isolates (Sample code)	Catalase test	Indole test	Methyl red test	VP test	Citrate utilization test	Glucose fermentation test	Gas production in glucose fermentation	Lactose fermentation test	Gas production in lactose fermentation
P-NA1	+	-	+	-	-	+ +	+	+ +	+
P-NA2	+	-	-	+	-	-	-	-	-
B-NA	+	+	+	-	-	+	-	+	-
BS-NA1	+	-	+	-	-	+	-	-	-
BS-NA2	-	-	-	+	-	+	-	+	-
P-MAC	+	-	-	+	+	+ +	-	-	-
O-MAC1	+	-	-	+	+	-	-	-	-
O-MAC2	+	-	-	+	-	-	-	-	-
O-MAC3	-	-	+	-	-	-	-	-	-
BS-MAC1	+	-	-	+	-	+	-	-	-
BS-MAC2	-	-	+	-	+	-	-	-	-
BS-MSA1	+	+	-	-	-	+	-	+	-
P-SMH	+	+	-	+	-	+ +	-	+	-

Conclusion

The present study concludes that different types of bacteria are present in wound samples. The different types of wound comprise of different bacterial isolates. The bacteria may bepathogenic, and if not taken into consideration they can cause serious health problems.

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