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SHORT COMMUNICATION

Microflora on the Surface of Laboratory Instruments

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Abstract

Microflora from the handle of B.O.D. incubator, handle of oven, handle of refrigerator, handle door of LAF room, weighing balance switch, fine adjustment switch of microscope, control knob of water bath, control knob of oven were isolated and identified. Microorganisms were isolated and characterized by standard methods. All swab containing tubes showed turbidity in broth tubes and further, biochemical tests confirmed that isolated strains were *Streptococcus pyogens*, *Staphylococcus aureus* and *S. epidermidis*. *Staphylococcus aureus* strains were collected from all samples sites but *S. epidermidis* strains were present only in handle of B.O.D. incubator and handle of door of LAF. *Streptococcus pyogens* was present in all samples except control knob of water bath and control knob of oven.

Keywords: Microflora, turbidity, biochemical tests, *Streptococcus pyogens*, *Staphylococcus aureus*.

Introduction

Microorganisms are responsible for communicable diseases and can spread from various objects. The opportunistic pathogens have ability to persist and multiply in a variety of environments and cause a wide spectrum of diseases in both humans and animals (Pilipcincova *et al.*, 2010; Akinkunmi and Lamikanra, 2010). *Staphylococcus* and *Streptococcus* bacteria are normal microflora of healthy humans but they are responsible for various diseases such as bacteremia and endocarditis, pneumonia, bone and joint infections and central nervous system (CNS) infections (Snider and Swedo, 2003; Liu *et al.*, 2011). Reservoirs of pathogens were identified in the faeces, around the ear and in the axilla and nares (Eastick *et al.*, 1996). Few reports indicated that these gram positive bacteria were present on various objects such as computer keyboards, door handles, tourniquets, pens, television sets, stethoscopes, telephones, beds and bedside tables, equipment packaging, paper and patient's notes (Oie and Kamiya, 2002; Panhotra *et al.*, 2005; Ciragil *et al.*, 2006).

Staphylococcus aureus is responsible for nosocomial infections that have come to prominence through the rise of drug-resistant forms, particularly methicillin-resistant *S. aureus* (MRSA) (Thwaites and Gant, 2011). Deshwal (2012) mentioned that *S. aureus* caused urinary tract infection (UTI). *Streptococcus pneumoniae* is the one of the most common cause of community-acquired pneumonia, meningitis, and bacteremia in children and adults (Lynch and Zhanel, 2009) and this normal microflora had the ability to cause disease. The present investigation is intent to isolate and characterize of the microorganism present on the surface of laboratory instruments.

Materials and methods

Isolation of microorganisms: Microorganisms were isolated from the handle of B.O.D. incubator, handle of oven, handle of refrigerator, handle of door of LAF room, weighing balance switch, fine adjustment switch of microscope, control knob of water bath, control knob of oven. Three sterile wet hi-media swab was spread on the surface of above said sites. One swab was transferred into nutrient broth, second swab was spread on nutrient agar medium, third swab was spread on Blood agar medium and incubated at 35±1^{o}C for 24 h.

*Characterization of isolated microorganisms***:** Isolated strains were characterized according to Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).
Haemolysis: Pure culture was transferred on blood agar plates and incubated at 35±2^{o}C and haemolysis was observed around the colony.
Growth at 45^{o}C: Pure bacterial culture was transferred in nutrient broth and incubated at 45^{o}C for 24 h and growth was observed in the medium.
Leucine aminopeptidase: Several log phase colony was transferred on moisten the LAP disk and incubated at room temperature for 5 min and a drop of cinnamaldehyde reagent was added. Development of a red/pink color with in 1 min after addition of cinnamaldehyde reagent showed positive results.
Bile-aesculine test: Pure culture was streaked on the bile aesculine agar slop and incubated at 35±2^{o}C for 5 d. Blackening showed positive results.
Pyrrolydonyl arylamidase: Filter paper disks were impregnated with L-pyrrolidonyl-β-naphthylamide (PYR). The colonies were picked up and inoculum was rubbed gently into the small area of the disk and incubated at room temperature for 2 min.

Color developer (1 mL) was added to the disk and allowed for 1 min for color change. A bright pink or cherry red color will appear within 1 min if the test is positive.

Phosphatase: Streak the pure culture on the Pikovskaya's agar plates and incubated at 35±2°C for 2-3 d. Clear zone around the streaking showed that strains produced phosphatase enzyme.

Pyridoxal or cysteine dependence: Membrane sterilized pyridoxal hydrochloride at a concentration of 0.001 g/100 mL or L-cysteine at a concentration of 0.01 g/100 mL was added separately in nutrient agar medium and plates were prepared. Pure culture was streaked on the agar medium and incubated at 35±2°C. The growth was observed after 24 h.

Other tests: VP test, hydrolysis of starch, H_2O_2, fermentation, oxidase test, coagulase test, nitrate reduction, arginine, urea hydrolysis were done according to Cowan and Steel's Manual for the identification of medical bacteria (Barrow and Feltham, 1993).

Sensitive to bacitracin (0.1 unit), 5 µg Optochin: Antibiotic sensitivity was done by disk diffusion method.

Yellow pigment: Streaked the pure culture on Nutrient agar plates and incubated at 35°C±2°C for 1-2 d and the color of the bacterial colony was observed. *Staphylococcus* strain produced yellow colony on NAM medium.

Growth anaerobically: Pure culture was streaked on Blood agar medium and anaerobic condition was maintained by candle jar method. Incubated at 35±2°C for 24 h and observed the growth.

Protease: Protease activity was done according to Lee *et al.* (1999).

Results

All swabs containing tubes showed turbidity in broth tubes. Further, biochemical tests confirmed that isolated strains were *Streptococcus pyogens*, *Staphylococcus aureus* and *S. epidermidis*. *Staphylococcus aureus* strains were collected from all samples sites but *S. epidermidis* strains were present only in handle of B.O.D. incubator and handle of door of LAF. *Streptococcus pyogens* was present in all samples except control knob of water bath and control knob of oven (Tables 1, 2 and 3).

Discussion

Gram staining confirmed that isolated strains were gram positive bacteria. Cocci in chain and grape like structure confirmed that isolated strains were Streptococci and Staphylococci respectively. Biochemical tests are essential for diagnosis of species of bacteria. Biochemical tests confirmed that isolated strains were *Streptococcus pyogens*, *Staphylococcus aureus* and *S. epidermidis*. Similar biochemical tests have been mentioned in Cowan and Steel's Manual for the identification of medical bacteria (Barrow and Feltham, 1993).

Table 1. Biochemical characterization of *Streptococcus pyogens*.

Biochemical tests	*Streptococcus pyogens*
Haemolysis	β
Growth at 45°C	-
Growth in 6.5% NaCl broth	-
Growth on 40% bile agar	-
Leucine aminopeptidase	+
Bile-aesculine test	-
VP test	-
Pyrrolydonyl arylamidase	+
Phosphatase	+
Pyridoxal or cysteine dependence	-
Hydrolysis of	
Hippurate	-
Aesculine	+
Starch	+
Sensitive to bacitracin (0.1 unit)	+
Optochin	-
H_2O_2 production	-
Fermentation of	
Pyruvate	-
Ribose	-
Arabinose	-
Mannitol	+
Sorbitol	-
Adonitol	-
Sucrose	+
Lactose	+
Trehalose	+
Raffinose	-
Insulin	-
Starch	+
Polysaccharide from sucrose	-
Motility	-
Yellow pigment	-

Table 2. Biochemical characterization of *Staphylococcus* strains.

Biochemical tests	*S. aureus*	*S. epidermidis*
Growth anaerobically	+	+
Oxidase	-	-
VP	+	+
Coagulase	+	-
Acid from		
Lactose	+	+
Maltose	+	+
Mannitol	+	-
Fructose	+	+
Sucrose	+	+
Trehalose	+	+
Xylose	-	-
Cellobiose	-	-
Raffinose	-	-
Mannose	+	+
Phosphatase	+	+
Nitrate	+	+
Arginine	+	+
Urea	+	+
Protease	+	+

Table 3. Isolation of microflora from various instruments and sites.

Sample sites	*Streptococcus pyogens*	*Staphyloccus* strains	
		S. aureus	*S. epidermidis*
Handle of B.O.D. incubator	+	+	+
Handle of Oven	+	+	-
Handle of refrigerator	+	+	-
Handle of door of LAF room	+	+	+
Weighing balance switch	+	+	-
Fine adjustment switch of microscope	+	+	-
Control knob of water bath	-	+	-
Control knob of oven	-	+	-

Similarly, Silva *et al.* (2000) reported results on the biochemical characteristics of *Staphylococcus* strains. Younis *et al.* (2000) studied the characterization of the phenotypic patterns of *Staphylococcus* strains and biochemical reactions. Some other reports are similar about biochemical characterization of *Staphylococcus* (Bjorkqvist *et al.*, 2002; Cunha *et al.*, 2004). Further, available literature suggested that *Staphylococcus aureus* was major pathogens which were present on major instruments. These strains are normal microflora of human body and therefore, *Staphylococcus* and *Streptococcus* were present on various sample sites. Recently, Ahmad *et al.* (2013) isolated staphylococci from hospital departments. Deshwal *et al.* (2011) isolated staphylococci from hospital personnel and hospital environment.

Conclusion

Incidence rate of *Staphylococcus* on various sites of instruments was highest as compared to other isolates. This may be that *Staphylococcus* is normal microflora of human body and is present on the figures tips. Authors suggested that periodic fumigation in microbiology lab is mandatory and care should be taken.

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