

There are various types of HAI reported in patients during their treatment such as hospital acquired pneumonia, majorly ventilator associated pneumonia, urinary tract infection, gastroenteritis, puerperal fever, central line-associated blood stream infections and others (Akbari *et al.*, 2015). Some studies suggest that most common organisms causing HAI are *Staphylococcus aureus*, *Methicillin resistant Staphylococcus aureus*, *Candida albicans*, *Pseudomonas baumannii*, *Stenotrophomonas maltophilia*, *Clostridium difficile*, *Escherichia coli*, *Vanomycin resistant Enterococcus* (Akbari *et al.*, 2015). The infection may be acquired by anyone admitted to a healthcare facility (Khan *et al.*, 2015). Stethoscope, sphygmomanometer and thermometer are the important instruments for medical professionals and are often used to assess the health of patients and the single medical instrument is often used for all patients. These instruments are the primary agents for the transmission of nosocomial infection. Furthermore sterilization of medical devices is not done in routine manner (Fenelon *et al.*, 2009). Though medical professionals were instructed about bacterial colonization and the importance of maintaining sterilized medical instruments, these devices may not be thought of a potential source of HAI (Wikins *et al.*, 2007). The use of 70% isopropyl alcohol and 90% ethanol was found to be effective in reducing bacterial colonization from medical equipments other than detergents etc. (Alothman *et al.*, 2009). Immune compromised persons are more prone to contract nosocomial infections.

Materials and Methods:

The samples were collected from the All India Institute of Medical Sciences (AIIMS), Patna. Total forty samples were collected from various instruments such as thermometer, sphygmomanometer and stethoscope from three

different departments namely Orthopedic, Pediatric and Gastroenterology by using a sterile ear buds (swab) soaked in a sterile saline. The present study was conducted in the Central Research Laboratory of Patna Women's College.

The sampling was done from the rim and diaphragm of Stethoscopes, rubber cuffs of Sphygmomanometers, bulb and expansion chamber of Thermometers, then the instruments were cleaned with 100 % ethanol and left for 4-5 minutes to act. The second samples were collected after the action of ethanol. The swab containing samples were stored in a transparent zip lock plastic pouches and carried to the Laboratory immediately. All the samples obtained were inoculated in MacConkey and Nutrient agar plates and incubated at 37°C for 24 hours. Isolation and characterization of the bacteria was carried out by initial morphological examination of the colonies grown on culture medium plate, wherein colour, texture and shape of the colony were recorded. Gram's staining and Biochemical tests such as Indole production test, Methyl red test, Voges-Proskauer test, Citrate utilization test and Catalase test were performed with standard protocol.

The Mueller-Hinton agar was prepared and the samples were spreaded to prepare lawn culture. The evaluation of the Ethanol, Isopropyl alcohol was done by Kirby-Bauer technique. Whatman filter paper of standard size were cut and sterilized separately in an autoclave at 121°C (15 lbs) for 15-20 minutes. Disks soaked in each concentration of different antibacterial solutions were kept on the surface of the lawn culture incubated at 37°C for 20-24 hours. The result was observed and zone of inhibition were measured for each one.

Results and Discussion:

In the present study we found thirty six (90%) isolated samples showed bacterial growth whereas

no growth was observed in rest of four (10%) samples. According to Bernard et al., 1999, stethoscopes used in hospitals contain high level of bacterial contamination. Further, bacterial isolates were characterized on the basis of colony morphology, gram's stain and biochemical assay wherein the shape, colour and texture of bacterial colony were studied by macroscopic observation as listed in Table 1 and Table 2. In our study we found that all the thirty six samples contained gram positive bacteria. The microscopic observations suggest that 11% of bacterial isolates were rod shaped and 89% were coccus shaped. Besides this we also found that the bacterial isolates from sphygmomanometer were coccus (11) and rod (2), stethoscope were coccus (10) and rod (2) and thermometer were coccus (11) and rod (0), our results are concurrent with the study reported by Shiferaw et al., (2013), where the gram positive cocci was isolated in more number of stethoscopes. On the basis of gram staining and biochemical assay we identified three bacterial strains on the equipments which are *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Listeria monocytogenes*. Similar to other report, we also found high frequency of *Staphylococcus aureus* on hospital equipments as compared to other bacterial strains shown in Figure 1 and 2. Bdareen (2009) showed dominant occurrence of *Staphylococcus spp.* on hospital equipments as compared to other species. We also found that all the instruments which we targeted for our study were contaminated with bacteria and frequency of bacterial isolates are listed in Figure 1, similarly we also calculated the percentage of each of the bacterial strain isolated from total sample studied as *L. monocytogenes* (11.1%), *S. epidermidis* (27.7%) and *S. aureus* (61.2%) (Figure 2).

Table 1. Morphological characterization of bacterial strain

Bacterial strain	Identification of colony			Gram stain
	Shape	Colour	Texture	
<i>Listeria monocytogenes</i>	Low convex	white	Moist	+ve
<i>Staphylococcus epidermidis</i>	Raised & Cohesive	Grey	Mucoid	+ve
<i>Staphylococcus aureus</i>	Circular	Yellow	Smooth & Shiny	+ve

Table 2. Biochemical identification of bacterial strain

Bacterial strain	Name of biochemical tests				
	IT	MRT	VPT	CIT	CAT
<i>Listeria monocytogenes</i>	-	+	+	-	+
<i>Staphylococcus epidermidis</i>	-	-	+	-	+
<i>Staphylococcus aureus</i>	-	+	+	+	+

Note: '-'= Negative, '+'= Positive; IT: Indole Test, MRT: Methyl Red Test, VPT: Voges Proskauer Test, CIT: Citrate Test, CAT: Catalase Test.

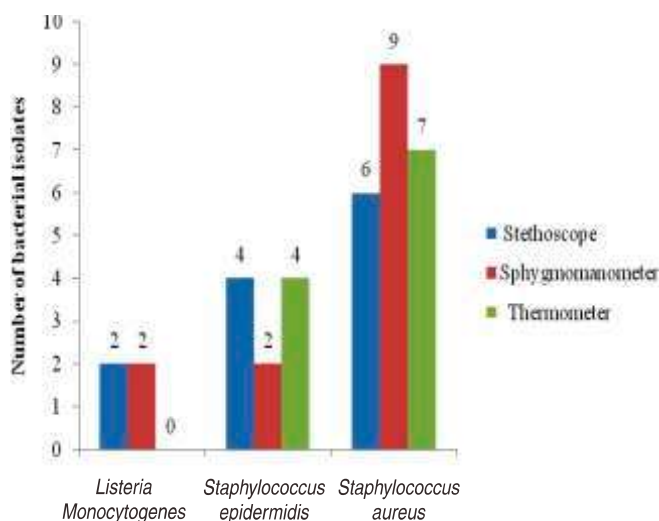


Fig. 1. Number of various bacterial strains isolated from medical equipments i.e. stethoscope, sphygmomanometer and thermometer.

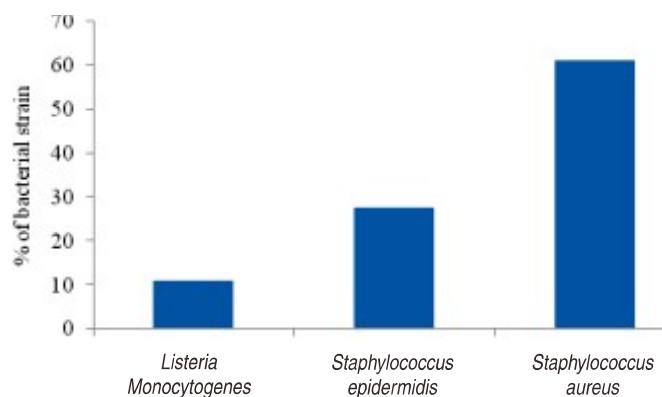


Fig. 2. Comparison in terms of percentage of various bacterial isolates obtained from different equipments. The data show that *L.monocytogenes* (11.1%), *S.epidermidis* (27.7%) and *S.aureus* (61.2%).

Further we did susceptibility tests of bacterial isolates with various concentrations of ethanol and we found minimal susceptibility for all the bacterial isolates even at highest ethanol concentration (Table 3). Our results again draw an important conclusion that only ethanol, treatment for sterilization of medical instruments is not good enough to check the nosocomial infections. Similar results were found with isopropyl alcohol even at 70% dose which is usually used for sterilization of majority of instruments used for diagnosis. (Table 4).

Table 3. Zone of inhibition with Ethanol against these bacterial isolates

SI. No.	Bacterial isolates	Ethanol concentration			
		25%	50%	75%	100%
1	<i>Listeria monocytogenes</i>	-	-	-	-
2	<i>Staphylococcus epidermidis</i>	-	-	-	6 mm
3	<i>Staphylococcus aureus</i>	-	-	-	-

Table 4. Zone of inhibition with Isopropyl Alcohol against these bacterial isolates

SI. No.	Bacterial isolates	Isopropyl concentration			
		10%	30%	50%	70%
1	<i>Listeria monocytogenes</i>	-	-	-	6.3 mm
2	<i>Staphylococcus epidermidis</i>	-	-	-	-
3	<i>Staphylococcus aureus</i>	-	-	-	-

Hence, the present study suggests standard protocol and repeated sterilization of medical instruments can minimize nosocomial infections.

Conclusion:

Present study showed that equipments such as Sphygmomanometer, stethoscope and thermometer can be potential carrier of infectious agents. In our study we found bacterial strains such as *Listeria monocytogenes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* which can be probable causative agents of hospital acquired infections. We found that samples collected from all the departments such as Orthopedic, Gastroenterology and Pediatric ward showed bacterial contamination with pathogenic importance. Among bacterial isolates *Staphylococcus aureus* (61.2%), *Staphylococcus epidermidis* (27.7%), and *Listeria monocytogenes* (11.1%) were observed. These bacterial isolates are reported to cause diseases such as blood borne infections, urinary tract infections, kidney infections and respiratory infections. It is further suggested that adherence to good infection control procedure particularly hand washing before and after interacting with patients. Hence we can conclude from our study that equipments used in hospitals are major source for carrier of bacteria having pathogenic importance and standard sterilization protocol is required to avoid hospital acquired infections.

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