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Research Article
Molecular Detection of Multidrug Resistance Pathogenic Bacteria from Protective Materials Used By Healthcare Workers (HCW); Bangladesh Scenario

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Abstract

Background and Objective: The increasing trend of Hospital acquired infections (HAIs), especially the ones caused by the multidrug resistant organisms has become a major public health concern. So that the aim of this study was to detect the dissemination of multidrug-resistant pathogenic bacteria on hands, gloves and masks of healthcare workers in some hospitals and pathology laboratories located in Noakhali and Dhaka, Bangladesh. **Materials and Methods:** A total of 106 fully fresh samples were collected. The samples were then subjected to various phenotypic cultural, biochemicals, antibiotic sensitivity along with molecular Polymerase Chain Reaction (PCR) analysis according to the standard procedures. **Results:** Approximately, 138 (57.5%) representative bacterial isolates were recovered among which the most frequently identified bacterium was *E. coli* 72 (52.17%) followed by *Staphylococcus aureus* 42 (30.43%), *Salmonella typhi* 14 (10.14%) and *Pseudomonas aeruginosa* 10 (7.25%). Among them total 33.33, 100 and 40% of *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* were pathogenic scanned through *hlg*, *inv*A and *Oprl* genes presence respectively. In contrast *E. coli* was tested through Congo red binding test where 36.11% were found pathogenic. The general frequency of Multiple Drug Resistance (MDR) pathogens were 79.31, 51.72, 68.97, 24.13, 6.89, 6.89, 17.24, 82.87, 24.13, 65.52 and 100% against ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, methicillin, streptomycin, tetracycline, nalidixic acid, doxycycline and imipenem consecutively. **Conclusion:** Healthcare personnel convey multiple drug resistance pathogenic bacteria in their protective materials which are potential source of nosocomial infections. Appropriate infection prevention measures, such as good hygiene practices and training for the healthcare workers should be taken to minimize the risks that are associated with the high rate of cross-contamination.

Key words: Multidrug resistant, pathogenic bacteria, antibiotic sensitivity, cross contamination and nosocomial infections

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Different types of microorganisms are hidden in the hospital environments among them some are pathogenic and play role in spreading Hospital-acquired infections (HAI)[1]. Pathogenic bacteria accountable for HAI include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococci*, *Acinetobacter* spp. and Coagulase-negative staphylococci. Some of them survive long time in the hospital environment naturally or through the formation of biofilms like *Pseudomonas aeruginosa* and *Staphylococcus aureus* and acquired resistance against commonly used disinfectants[2]. The ability of the bacterial population to resist antibiotics and disinfectants help them to reach patients through different sources and cause nosocomial infections[3].

In hospitals different types of precautions are taken by the healthcare workers to prevent the transmission of harmful bacteria to the patients. That is why; hand gloves and mouth masks are used. Improper implementation of hand washing practices of HCW is still a barrier to achieve the expected outcome of the taken precautions [4]. Thus, contaminated hand gloves, masks and even mobile phones are playing important in spreading multidrug resistant pathogenic bacteria[5]. HAIs normally occur after two days of staying in the hospitals[6]. HAIs are of major concerns as it leads the mortality and morbidity of the hospitalized patients greatly but one-third of such infections can be prevented by precise actions [7]. Hands as well as gloves of HCWs are responsible for the spread of pathogenic bacteria causing nosocomial infections. The presence of antimicrobial resistant bacteria on contaminated gloves and hands of HCWs in hospitals poses a threat to public health[8].

Multidrug resistant (MDR) pathogenic bacteria for instance methicillin-resistant *S. aureus* (MRSA), broad spectrum beta-lactamase producing Enterobacteriaceae, ceftazidime-resistant *P. aeruginosa*, imipenem-resistant *A. baumannii* and vancomycin-resistant *Enterococci* are generally chance meeting in the healthcare facilitates[9,10]. As Bangladesh is a developing country, the rate is HAIs in Bangladesh is very high due to inadequate judgments, ignorance and proper consciousness[11]. Antimicrobial resistance pattern of HAIs related bacteria have changed a lot in the last few years and very few studies have been reported in this regards [12, 13]. Moreover, the data related to it are not up to date in Bangladesh and thus are not reliable[14]. That is why; it examined the current condition of bacterial contamination of hands, hand gloves and mouth masks of HCWs and evaluated the pathogenicity and antimicrobial resistance pattern of isolated bacteria. Though the govt. is increasing the supervision system to control the nosocomial infections but the presence of multidrug resistance pathogenic bacteria and new emergence of pathogenic bacteria are increasing terribly. That is why, the study aimed to recover the dissemination of pathogenic bacteria on hands, gloves and masks of healthcare workers in some hospitals and pathology laboratories located in Noakhali and Dhaka and determination of their pathogenicity and antimicrobial resistance pattern.

MATERIALS AND METHODS

Study design and initial processing: This study was accompanied within April 2017 and February 2018 at Noakhali (22.828973, 91.098944) and Dhaka (23.739944, 90.393575) regions in Bangladesh. A total 240 samples were taken from the healthcare worker of different hospitals and pathology centers. This sample includes gloves, surgical masks and direct Handler swab from HCWs. All the samples were transported to the laboratory using Nutrient agar (Oxoid, Basingstoke, UK) under refrigerated conditions and microbiological analyses were carried out immediately.

Isolation and identification of bacteria: For the primary isolation of bacterial population different selective and differential media for instance Salmonella-shigella (SS) agar, Mannitol salt agar (MSA), Cetrimide agar and Eosin Methylene Blue agar (EMB) media for *Salmonella*, *Staphylococcus*, *Pseudomonas* and *E. coli* were used respectively. Each suspected isolate was examined for Gram's staining and followed by inoculation into aforementioned biochemical test such as oxidase test, indole test, urease test, citrate test, Triple Sugar Iron (TSI) test, catalase and MR-VP were performed according to the guideline of the Bergey's Manual of Determinate Bacteriology[15].

Preparation of template DNA: The DNA of each selected isolate was prepared using freshly cultured bacterial colonies on nutrient agar plates, suspended in 150 µL of sterile distilled water in a micro centrifuge tube, gently vortexed and boiled for 10 min in a water bath. After centrifugation at 10000 rpm for 5 min at room temperature (20°C), the supernatant was immediately used for PCR reactions[13]. The extracted DNA concentration was determined by using Nano Drop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE)[16].

Screening of virulence genes to detect pathogenicity: Various individual pathogenic tests were performed for each isolated bacteria. For *E. coli* Congo red binding test was

performed as it is associated with the pathogenicity of *Escherichia coli*[17]. *inv*A, *hlg* and *Oprl* can explain the viluencity in *Salmonella*, *Staphylococci* and *Pseudomonas* respectively (Table 1). Recommended primer set as per Rahn *et al.*[18], Kumar *et al.*[19] and Fazeli and Momtaz[4] accordingly were utilized to amplify the specific gene in thermal cycler under specific PCR conditions for *Salmonella*, *Staphylococci* and *Pseudomonas*, respectively. The reaction was set as initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, extension at 72°C for 1 min and final extension was done at 72°C for 7 min. Subsequently, the amplified PCR product was visualized by agarose gel-electrophoresis (1.5% agarose gel).

Anti-biogram test: Disk diffusion method was used to evaluate the antimicrobial resistance pattern of the bacterial isolates [20]. Fresh Bacterial inoculums having turbidity of a . 1-.5 nm using spectrophotometer. The antimicrobial susceptibility testing was performed using Mueller-hinton medium against ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), methicillin (5 µg), streptomycin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), Doxycycline (30 µg), Imipenem (10 µg). The plates were incubated aerobically at 37°C for 18-24 h. The zones of inhibition were measured as stated by Clinical Laboratory Standards Institute (CLSI)) guidelines[21].

RESULTS

The present study focused on isolation of multidrug resistant and pathogenic bacteria mainly *E. coli*, *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa* in hands, gloves and masks of healthcare workers in some hospitals and pathology laboratories located in Noakhali and Dhaka, Bangladesh.

Bacterial isolation and presumptive identification: From all samples, initially 138 isolates with characteristic colonies were detected positive based on the colony characteristic on SS Agar, MSA, Cetrimide agar and EMB Agar. For presumptive identification of *E. coli, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa*. A panel of biochemical test (IMViC, TSI and Urease) was performed. Out of 240 samples, after completing all biochemical tests, 138 (57.5%) isolates were presumptively identified. Among all isolates, 42 (30.43%) were *Staphylococcus*, 10 (7.25%) *Pseudomonas*, 14 (10.14%), *Salmonella* followed by 72 (52.17%) *Escherichia coli*. Overall, a high prevalence of pathogenic bacteria was reported in Handler swab samples where the percentage was 49.28. Bacteria were isolated in all the abattoir types; the most prevalent bacteria were *E. coli* while *Pseudomonas* was the lowest. No *Salmonella* was detected in this study from face mask samples (Table 2).

Screening of virulence character to detect pathogenicity: Overall, pathogenic *E. coli* was detected in 26 (36.11%) congo red binding test. In contrast 14 (100%), 33.33% (14) and 40% (4) pathogenic *inv*A, *hlg*, *Oprl* genes were detected in *Salmonella typhi, S. aureus* and *Pseudomonas aeruginosa* respectively in this study (Fig. 1).

Table 1: Primers used for the detection of *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa* by PCR

Genes	Sequences (5'-3')	Size (bp)	Tm value	References
invA	GTGAAATTATCGCCACGTTCGGGCAA			
	TCATCGCACCGTCAAAGGAACC	284	58°C	Momtaz *et al.*[13]
Hlg	GCCAATCCGTTATTAGAAAATGC			
	CCATAGACGTAGCAACGGAT	937	55°C	Kumar *et al.*[19]
Oprl	ATGAACAACGTTCTGAAATTCTCTGCT			
	TTGCGGCTGGCTTTTTCCAG	249	55°C	Fazeli and Momtaz[4]

Virulence genes (A) *hlg* (937 bp), (B) *invA* (284 bp) and (C) *Oprl* (249 bp) were used for *Staphylococci*, *Salmonella* and *Pseudomonas*, respectively

Table 2: Bacteria distribution in the different Samples of HCWs examined in this study

Samples		Bacterial distribution in the different samples of HCWs					
Source of samples	No. of samples	*Salmonella*	*E. coli*	*S. aureus*	*Pseudomonas*	Prevalence in the samples (%)	Total No. of positive bacteria
Gloves	80	4 (9.09%)	26 (59.09%)	12 (27.27%)	2 (4.55%)	31.88	44
Face mask	80	0 (0.00%)	12 (46.15%)	12 (46.15%)	2 (7.7%)	18.84	26
Handler swab	80	10 (14.71%)	34 (50%)	18 (26.47%)	6 (8.82%)	49.28	68
Total	240	14 (10.14%)	72 (52.17%)	42 (30.43%)	10 (7.25%)	100%	138
Pathogenic	138	14 (100%)	26 (36.11%)	14 (33.33%)	4(40%)	42.03	58

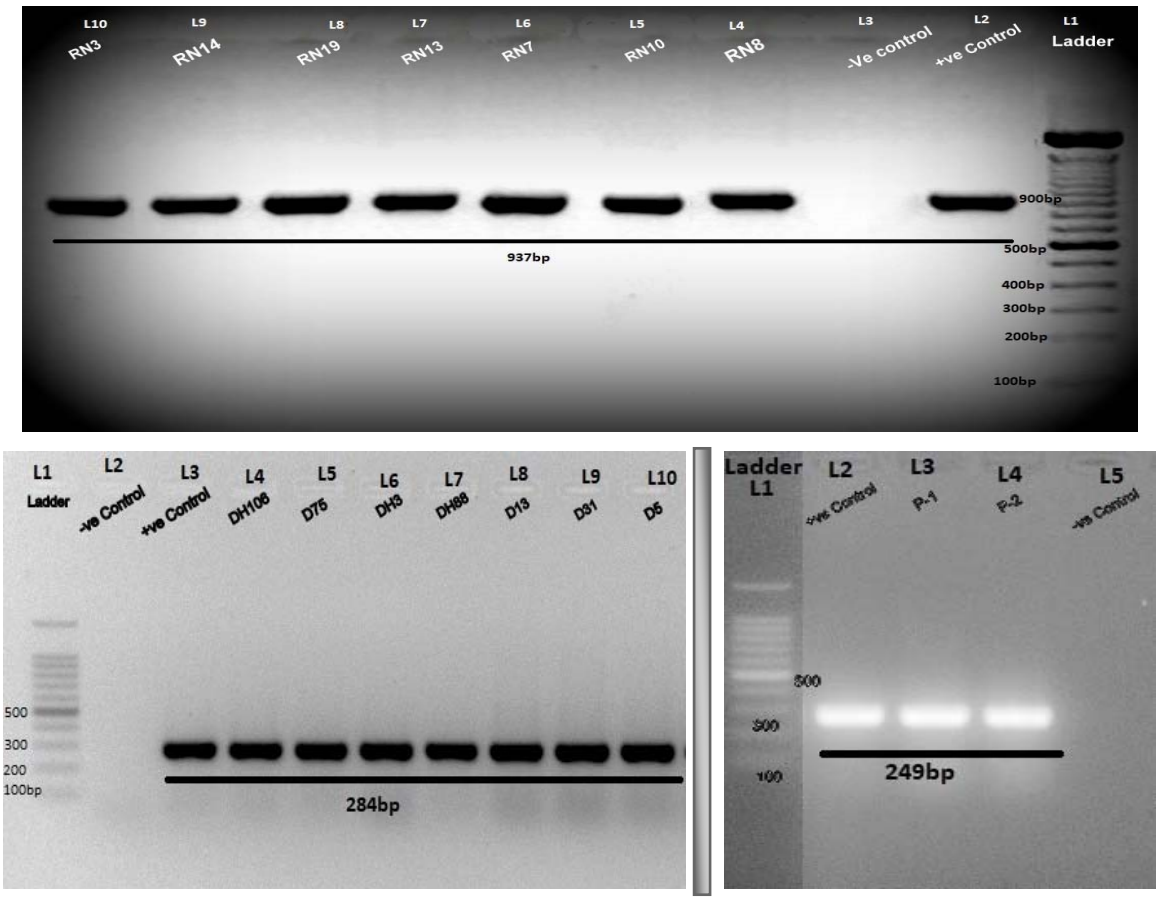

Fig. 1(a-c): Amplification products of virulence genes: (a) *hlg* (937 bp) for *Staphylococci*, (Lane 1: 100 bp ladder as molecular size DNA marker. Lane 2: +ve control, Lanes 3: -ve control, Lane 4: 10 represent group (*Staphylococci*)), (b) *invA* (284 bp) for *Salmonella,* (Lane 1: 100 bp, Lane 2: +ve control, Lanes 3: -ve control, Lane 4: 10 represent group (*Salmonella*)) and (c) *Oprl* (249 bp) for *Pseudomonas,* (Lane 1: 100 bp, Lane 2: +ve control, Lanes 3: -ve control, Lane 4: 10 represent group (*Pseudomonas*))

Antimicrobial susceptibility testing: It conducted antibiogram profile for all pathogenic isolates. Out of the 14 isolated *S. aureus*; 14 (100%), 10 (71.43%), 8 (57.14%), 14 (100%), 4 (28.57%), 10 (71.43%), 10 (71.43%) and 14 (100%) isolates were resistant to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, methicillin, streptomycin, tetracycline, Imipenem. Similarly, out of the 6 isolated *P. aeruginosa*, 4 (66.67%), 4 (66.67%), 2 (33.33%), 2 (33.33%), 4 (66.67%), 4 (66.67%) isolates were resistant to ampicillin, chloramphenicol, ciprofloxacin, tetracycline, doxycycline, imipenem. In total 18 (69.23%), 18 (69.23%), 12 (46.14%), 22 (84.61%), 22 (84.61%) and 26 (100%) isolates of *E. coli* were resistant to ampicillin, chloramphenicol, ciprofloxacin, tetracycline, doxycycline and imipenem, respectively. Out of the 14 isolates of *Salmonella typhi* 10 (71.43%), 8 (57.14%), 6 (42.86%), 4 (28.57%), 14 (100%), 14 (100%), 12 (85.71%), 14 (100%) isolates were resistant to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, nalidixic acid , doxycycline and imipenem (Fig. 2). Finally, we also estimated the total isolated bacteria among them 79.31, 51.72, 68.69, 24.13, 6.89, 6.89, 17.24, 82.72, 24.13, 65.52 and 100% isolates were resistant to Ampicillin, Chloramphenicol, Ciprofloxacin, Erythromycin, Gentamicin, Methicillin, Streptomycin, Tetracycline, Nalidixic acid, Doxycycline, Imipenem antibiotics, respectively (Fig. 3, Table 3).

DISCUSSION

Due to the lack of insufficient research on the degree of bacterial infections in the hospitals mainly on healthcare and pathology centers' workers, yearly medical expenditures going up in the healthcare facilitates. Not only that, the healthcare facilitates are opening infection management system because of various unwanted infectious. For the dramatic increase of

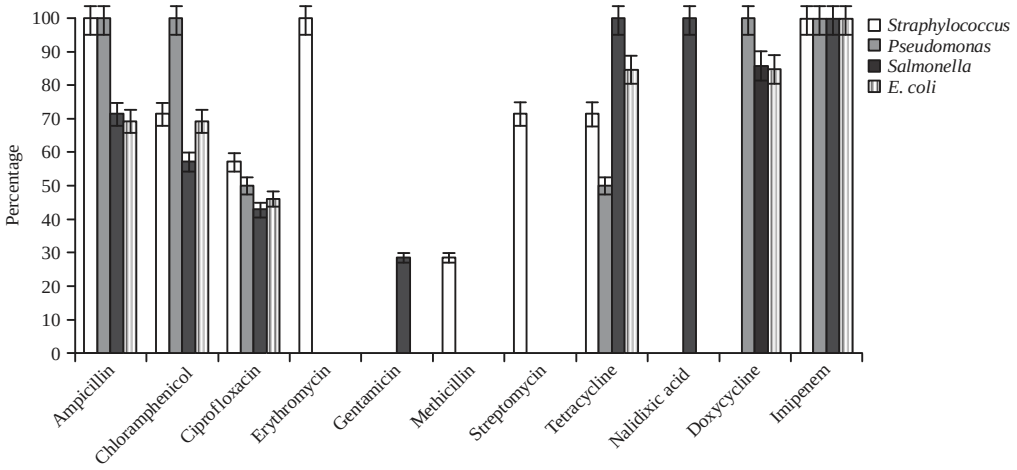

Fig. 2: Percentage of resistance of isolated microorganisms against specific antibiotics

Table 3: Antibiogram pattern of isolated pathogenic bacteria

Antibiotics	Sensitivity pattern	*S. aureus*	*P. aeruginosa*	*S. typhi*	*E. coli*
Ampicillin	R	14	4	10	18
	I	0	0	4	2
	S	0	0	0	6
Ciprofloxacin	R	8	2	6	4
	I	2	2	4	2
	S	4	0	4	4
Chloramphenicol	R	10	4	8	18
	I	0	0	4	0
	S	4	0	2	8
Erythromycin	R	14	NA	NA	NA
	I	0			
	S	0			
Gentamicin	R	NA	0	4	0
	I		0	0	2
	S		4	10	24
Methicillin	R	4	NA	NA	NA
	I	4			
	S	6			
Streptomycin	R	10	NA	NA	NA
	I	2			
	S	2			
Tetracycline	R	10	2	14	22
	I	4	2	0	0
	S	0	0	0	4
Nalidixic acid	R	NA	NA	14	NA
	I			0	
	S			0	
Doxycycline	R	NA	4	12	22
	I		0	0	2
	S		0	2	2
Imipenem	R	14	4	14	26
	I	0	0	0	0
	S	0	0	0	0

NA: Not applicable, R: Resistance, I: Intermediate, S: Sensitive

hospital infections recently various important accessories like clothes, hands and mobile phones of healthcare workers for instance doctors, specialists and nurses were inspected. The consequences revealed that healthcare workers' mobile phones, wear uniforms healthcare workers' gowns, gloves and shirt sleeves possible means of bacterial dissemination between patients and hospitals personnel[22-26]. So far in our country, there are not adequate researches on the level of

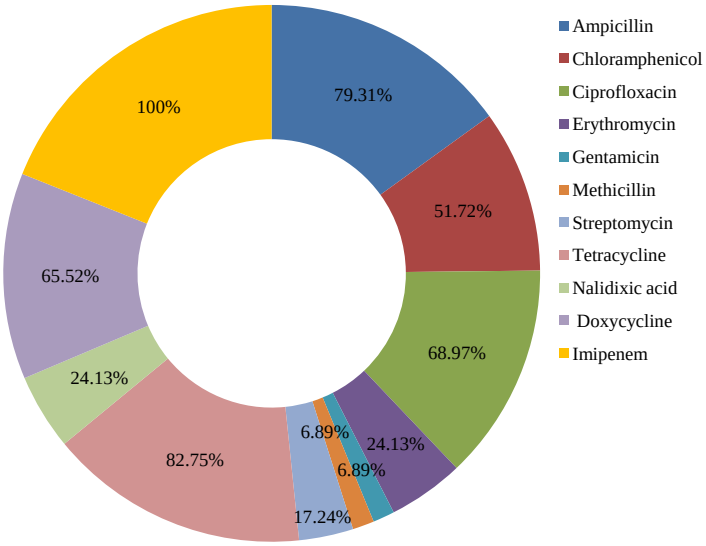

Fig. 3: Antibiotic resistance pattern of isolated microorganism against different antibiotics

bacterial infections especially targeting the healthcare workers' accessory materials. That is why, it projected to recover pathogenic bacteria from hands, mask and hand swab samples of hospital and pathology workers.

As a result of isolation of pathogenic bacteria from hospitals and pathology centers workers' hands, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Salmonella typhi* were isolated where 49.28% were from Handler swab samples, 31.88% were from gloves swab samples and 18.84% were from masks. The rate of isolation of bacterial pathogens in the handler swab samples was higher than the face mask samples. Similar trends was reported by Chaka *et al.*[27], Hall *et al.*[28] and Luksamijarulkul *et al.*[29] where bacteria were isolated from hands, gloves and other accessories of healthcare workers and wherein 78, 81.1, 15±9 CFU mL^{-1}/piece and 61.53% of hand swabs, gloves, mask and female fingernails were contaminated respectively.

Multiple drug resistance was also usual in Gram-negative and Gram-positive bacterial isolates to commonly used antibiotics in these study areas. *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Salmonella typhi* were 100% resistant to imipenem and also resistance to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, methicillin, streptomycin, tetracycline, nalidixic acid, doxycycline in various ranges which is consistence with the study of Rocha *et al.*[1] who also recovered these four types of bacteria to be multidrug resistance in different levels. In the case of *S. aureus*, got only 2 MRSA phenotypically which is similar to Kim and Jeong[30] who also found 2 strains of MRSA from 104 samples of dentists depending on the presence of *mec*A gene.

According to the discussion, it can be considered that the possible source of bacterial infection from hospital system could be originated from healthcare workers' accessories. Moreover, environment as a source for the chance of infection could not be declined, as prevalence of drug resistance bacteria is a common scenario in Bangladesh in environmental effluents. The resistance pattern observed is also a huge challenge in treating infectious diseases with the commonly available drugs. Despite a relatively high level of knowledge about the potential role of examined samples as bacterial reservoirs, the habits of hygienic practices were very poor. This poor hygiene may help to disperse the MDR bacteria to the hospitalized patient as nosocomial infection which is a great threat now a day. So, regular cleaning and disposal by health care workers and implementation of appropriate infection prevention guideline will help in reducing the possible risks associated with those accessories of health care workers and reduce rate of nosocomial infection.

SIGNIFICANCE STATEMENT

This study discovers the sources of MDR bacteria from the hospital environment that can be beneficial for the patient who has admitted into the hospital and getting various lives threading nosocomial infection. Due to nosocomial infection which they have got after hospital admission every year numerous people suffer and even died due. Hospital is multidimensional environments where lots of patients come with various types of disease. So there are lots of chance to

spread out contagious diseases and pathogenic bacteria from one person to another in the hospital. But knowing the sources of spreading disease one can easily prevent this spreading lots of factors may play role in this whereas HCW is one of the major as they come close enough to the patient. So by maintaining proper's hygiene about their protective material nosocomial infection rate can be reduced at such a significant rate. This study will help the researcher to uncover the critical areas contributes to the spreading of pathogenic bacteria that many researchers were not able to explore. Thus a new theory on reducing nosocomial infection as well as pathogenic MDR bacteria dispersal may be arrived at.

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