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Comparison Between Reduced Susceptibility to Disinfectants and Multidrug Resistance Among Hospital Isolates of *Pseudomonas aeruginosa and Staphylococcus aureus in Bangladesh*

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ABSTRACT

Disinfectants have been used largely in hospitals, health care centers and different pharmaceuticals for the removal of microorganisms. It is evident that microorganisms are showing reduced sensitivity against many disinfectants or their minimum inhibitory concentration (MIC) is increasing day by day due to improper use. The aim of this study was to compare the reduced susceptibility to disinfectants and antibiotics of 25 hospital isolates of *Pseudomonas aeruginosa* and 40 hospital isolates of *Staphylococcus aureus* isolated from 5 different hospitals at Noakhali region of Bangladesh. Susceptibility of the selected isolates to two disinfectants (savlon and herpic) and ten separate antimicrobial agents for both *P. aeruginosa* and *S. aureus* were investigated and compared. Multidrug resistant pattern of all the hospital isolates were determined by agar diffusion method and MIC of the disinfectants were determined by the serial dilution method. All the hospital isolates of *P. aeruginosa* and *S. aureus* were multidrug resistant. No severe evident resistance to disinfectants was seen among the 25 isolates of *P. aeruginosa* and 40 isolates of *S. aureus*. Interestingly, satisfactory MIC of savlon for 25 isolates of *P. aeruginosa* and 40 isolates of *S. aureus* reached at 0.5% to 0.7% (v/v) solution whereas satisfactory MIC of herpic reached at 2% to 2.5% (v/v) solution for all hospital isolates but four isolates of *S. aureus* showed MIC against herpic at 1.75% (v/v) solution. No sign of co-resistant of disinfectant and antibiotics were found. So, it can be concluded that disinfectants (savlon and herpic) can't be responsible for *P. aeruginosa* and *S. aureus* to become multidrug resistant, when the semi inhibitory dilution of these disinfectants are used.

Keywords: disinfectants, multidrug resistant, *P. aeruginosa, S. aureus*

ÖZET

Bangladeş'teki hastanelerde toplanan *Pseudomonas aeruginosa* ve *Staphylococcus aureus* izolatlarında bulunan dezenfektanlara karşı azaltılmış duyarlılık ve çoklu ilaca direnç gelişimi arasında karşılaştırma

Amaç: Mikroorganizmaların giderilmesini sağlamak amacıyla hastaneler ve sağlık tesislerinde dezenfektanlar ve çeşitli tür ilaçlar kullanılmaktadır. Bilindiği gibi uygunsuz kullanımdan dolayı mikroorganizmaların dezenfektanlara karşı gösterdiği duyarlılık azalıyor ya da minimum engelleyici konsantrasyonu (MEK) günbegün artıyor. Çalışmamız, Bangladeş'in Noakhali bölgesinde bulunan 5 hastaneden alınan 25 tane *Pseudomonas aeruginosa* ve 40 tane *Staphylococcus aureus* hastane izolatlarında, dezenfektanlara ve antibiyotiklere karşı gelişen azaltılmış direnç derecelerini karşılaştırmayı amaçladı. Seçilmiş olan *P. aeruginosa* ve *S. aureus* izolatlarının iki dezenfektan (Savlon ve Harpic) ve on tane farklı antimikrobiyal ajana karşı gösterdiği duyarlılıklar araştırılıp karşılaştırıldı. Bütün hastane izolatlarının çoklu ilaca direnç kalıpları, agar diffüzyon yöntemi kullanarak saptanıp dezenfektanların MEK'leri seri seyreltme yöntemiyle bulundu. Hem *P. aeruginosa* hem de *S. aureus*'un bütün hastane izolatları çoklu ilaca dirençli bulundu. Ne 25 *P. aeruginosa* izolatında ne de 40 *S. aureus* izolatlarında, şiddetli açık dezenfektanlara karşı direnç bulundu. İlginçtir ki, Savlon'un yeterli MEK'si hem 25 *P. aeruginosa* izolatı hem 40 *S. aureus* izolatı için %0,5 ile %0,7 (hacimsel oran) aralığında seyrederken, Harpic'in MEKsi bütün hastane izolatları için %2 ila %2,5 (hacimsel oran) aralığında bulundu. Ancak *S. aureus*'un dört tane izolatı Harpic'e karşı %1,75 (hacimsel) solüsyonda MEK'ye ulaştı. Dezenfektan ve antibiyotiklere karşı gelişen eş zamanlı direncin hiçbir belirtisi bulunmadı. Özetle, engelleyici konsantrasyonla kullanılan dezenfektanların (Savlon ve Harpic) *P. aeruginosa* ve *S. aureus*'un çoklu ilaca direnç kazanımı için sorumlu tutulması mümkün değil.

Anahtar kelimeler: dezenfektanlar, çoklu ilaca direnç, *P. aeruginosa, S. aureus*

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Introduction

P. aeruginosa is an opportunistic pathogen and responsible for hospital-acquired infections (1,2). *P. areuginosa* is a virulent, antibiotic resistant and sometime resists disinfectants. That's why effective antibiotics are limited for them (3,4). On the other hand, the disinfectant solutions that are contaminated with pathogenic microbes may be the sources of hospital related infections (5,6). Methods that contrive to lower the microbial load and presence of causative agents of nosocomial infections include chemotherapy, immunization, sterilization and disinfection (7). Besides, in any infection control program disinfection, decontamination and sterilization are the main ways of control (8,9). The formulation, uses, storage condition and examination procedures of disinfectants interfere with the evaluation of their activity (10). Disinfectants act on bacteria by a variety of mechanisms including up taking the disinfectant by the cell (11), breaking the cell membrane leading leakage of intracellular materials (12), perturbation of cell homeostasis (13), effects on model membranes (14), inhibition of biochemical processes of the cell (15,16). *S. aureus* are encyclopedic colonizers that colonize on human and animal skin as well as mucous membranes and causes a variety of hospital and community-acquired infections that range from mild symptoms including skin and soft tissue infections to severe life-threatening sepsis (17). Methicillin-Resistant *S. aureus* (MRSA) strains are continuing to be a serious nosocomial infectious agent which were first introduced in healthcare centers. But it is also true that methicillin sensitive *S. aureus* (MSSA) may also be responsible for such hospital acquired infections (18). As MRSA infections are mainly related to the hospitals, to reduce the risk in the hospitals proper implementation of good hygienic procedures and surveillance program are very important (19).

Antiseptics and disinfectants have been used largely in hospitals, healthcare facilitates and different pharmaceuticals for the removal of microorganisms from different medical instruments. Moreover, biocides play an important role in infection control. They also play role in the control of microorganisms that are related to hospital acquired infections (20). The effect of different biocides in the prevention of contamination by various microorganisms have been described, (16) on the other hand reducing susceptibility to disinfectants has been found for some nosocomial pathogens, for example *Acinetobacter baumannii* (21,22), *Pseudomonas stutzeri*. Moreover decreased susceptibility to biocides was evaluated in another study with clinically isolated *Acinetobacter* spp. but no apparent development of resistance to disinfectants was found (22-24). Recently, it was reported that repeated use of semiinhibitory dilutions of some disinfectants reduced the susceptibility of some microorganisms to that disinfectants (25). Comparison between the degree of multidrug resistance and MICs of disinfectants was evaluated (16,25). On the other hand, same results were observed for other hospital related pathogens, such as MRSA and *P. aeruginosa* (25). However, the available information about this linkage are limited to a few bacterial species. *P. aeruginosa* and *S. aureus* have been becoming multidrug resistant for the last 10 years rapidly. That's why clear understanding of susceptibility to disinfectants among the hospital isolates of *P. aeruginosa* and *S. aureus* with available information will help to draw a correlation with the reduced susceptibility to antibiotics that may be helpful in controlling the hospital related infection.

Materials and Methods

Selection of Sample Collection Points

The sample collection points were mainly General Hospital, Prime Hospital, Royal Hospital, Apollo Hospital and Good heal Hospital in Noakhali region of Bangladesh. The samples were collected between Augusts to September, 2016. The study samples mainly included the swab of floors and utensils (beds, trays, equipment in Operation Theater), where disinfectants were used. The study samples were also collected from drains related to these hospitals. Total 100 samples were collected of which 50 samples were collected from different instruments and equipment, 30 samples were collected from floors of hospitals and the remaining 20 samples were collected from the drains related to the hospitals mentioned above. Among them, 10 instrumental and equip mental samples, 6 floor swabs and 4 drain samples were collected from each institute.

Procedure of Sampling

Each sample taken by swab was collected using sterile cotton butt and immediately inoculating into sterile nutrient broth. All the samples were taken to the laboratory within three hours. The drain samples were taken by sterile syringe and used for examination by dilution. The nutrient broth was kept for four hours for enrichment.

Bacteriological Investigation

For initial screening of *Staphylococcus aureus* from the collected samples, a loop of each sample was inoculated onto C.L.E.D. agar medium, mannitol salt agar medium, baired-parker agar and blood agar medium and incubated at 37°C for 24 hours. After the confirmation from cultural methods, all positive isolates of *S. aureus* were subjected for different biochemical tests such as catalase test, gram staining, coagulase test, oxidase test according to Bergeyes's Manual of Bacteriology (26). For initial screening of *P. aeruginosa* from the collected samples, a loop of each sample was inoculated onto C.L.E.D. agar medium, MacConky agar medium, Cetrimide agar medium and incubated 37°C for 24 hours. After the confirmation from cultural methods, all positive isolates of *P. aeruginosa* were subjected for different biochemical tests such as catalase test, gram staining, coagulase test, oxidase test, citrare test according to Bergeyes's Manual of Bacteriology (26).

Detection of *P. aeruginosa* and *S. aureus* by Genotypic Method (PCR)

The primer (gyr. b190) specific to *P. aeruginosa* were chosen from the published sequence of Lee et al. and Qin et al. (27,28). The 3'-end of *P. aeruginosa* specific gene was amplified using an18nucleotideforwardprimer5'-GGCGTGGGTGTGGAAGTC -3' and a 22 nucleotide reverse primer, 5'-TGGTGGCGATCTTGAACTTCTT-3', respectively. *P. aeruginosa* specific gene has the amplicon size of 190. The 3'-end of *S. aureus* specific gene was amplified using a 30 nucleotide forward primer 5'-AATCTTTGTCGGTACACGATATTCTTCACG -3' and A30 nucleotide reverse primer, 5'-CGTAATGAGATTTCAGTAGATAATACAACA-3' (which hybridize to 5-34 and (112-83), respectively. *S. aureus* specific gene has the amplicon size of 107 using primers described by Martineau et al. (29).

DNA extraction

DNA was extracted from the bacterial isolates using DNA extraction method followed by Stegger et al. (30). DNA samples were stored at -20°C.

Preparation of Reaction Mixture

The reaction mixture for PCR was prepared by mixing the specific volume of the components in an appropriate sized tube where 7.5µl master mix, 0.5µl of forward primer, 0.5µl of reverse primer, 5.5µl of nuclease free water and 1 µl of template DNA were mixed to make a final reaction volume of 15µl. In all PCR, a negative control that contained no DNA template but all other components of the reaction was included. In relevant cases, a positive control that contained known DNA template carrying known gene was also included.

PCR parameters

For the detection of *S. aureus* PCR reaction was optimized with the following parameters: an initial denaturation step at 94°C for 5 min was followed by1 cycle of amplification this was followed by denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s ending with a final extension step at 72°C for 5 min. In case of *P. aeruginosa* PCR reaction was optimized with the following parameters: an initial denaturation step of 94°C for 5 min; a denaturation step of 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min; and a final extension step of 72°C for 10 min. 35 serial cycles of reaction was performed.

Electrophoresis

5-8µl of the PCR products were run in parallel with a 100bp Ladder molecular weight marker on a 2% agarose gel in TBE 1X. At the end of the reaction, the gel was taken in the Trans illuminator under UV light to take picture.

Antimicrobial Susceptibility Test

10 types of antibiotic discs were used for each type of isolates. The antibiotic discs used for *S. aureus* are Azithromycin – AZM 15, Tetracycline – TE 30, Gentamycin – CN 10, Ciprofloxacin – CIP 05, Cefotaxime– CAZ 30, Chloramphenicol – C 30, Cefoxitin– FOX 30, Sulphametho+ Trimetho – SXT 25, Ceftriaxone – CTX 30 and Oxacillin– OX 01. In case of *P. aeruginosae* Chloramphenicol – C 30; Ampicilin – AMP 10; Imipenem – IPM 10; Netilmicin – NET 30; Ciprofloxacin – CIP 05; Ceflazidime – CAZ 30; Gentamicin – CN 10; Trimethoprim-Sulfamethoxazole - SXT 25; Amikacin – AK 30; Azithromycin – AZM 15; Ceftriaxone – CRO 30 were used for confirmation of MDR.

Disinfectants used

Mainly two types of disinfectants were used to complete the study, Savlon and Herpic. The active ingredient of Herpic is

hydrochloric acid (10%) and butyl oleylamine as well as others in an aqueous solution that is why a very active disinfectant. Savlon Antiseptic Liquid contains Cetrimide 3.0% w/v and Chlorhexidine Gluconate 0.3% w/v. Also contains: Isopropyl alcohol, terpineol, liquid deodoriser, benzyl benzoate, d-gluconolactone, sodium hydroxide and purified water.

Microbial resistance testing of coagulase positive *S. aureus* and *P. aeruginosae* for confirmation of MDR (multi-drug resistant)

A bacterial turbidity equivalent of 0.5 McFarland standards was used as inoculum for each isolate. The antibiotic resistance pattern for the panel of antibiotics was determined by disc diffusion method considering the zone of inhibition sizes for each of the antibiotics as "resistant (R)", "intermediately resistant (I)", and "sensitive (S)" against the test isolates as recommended by the Clinical and Laboratory Standard institute (CLSI, 2007).

Microbial resistance testing of coagulase positive *S. aureus* for phenotyphic confirmation of MRSA

MRSA was determined phenotypically by a disk diffusion method on Mueller -Hinton agar (Oxoid) using methicillin (oxacillin and cefoxitin) resistance according to the Clinical Laboratory Standards Institute (formerly NCCLS) standards. Bauer -Kirby disk-diffusion procedure was used on Muller -Hinton (MH) agar containing 2% NaCl. A bacterial turbidity equivalent of 0.5 McFarland standards was used as inoculum for each isolate.

MIC (Minimal Inhibitory Concentration)

The MIC (Minimal Inhibitory Concentration) was determined by serial dilution method. 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1% v/v solution of savlon and 1.25%, 1.5%, 1.75%, 2%, 2.25% and 2.5% solution of herpic were prepared in nutrient broth. The Minimal Inhibitory Concentration was determined by the lowest concentration solution of the disinfectants that inhibited the growth of the concern isolates.

Results

Proportion of samples positive for *P. aeruginosa*

Among the 100 samples, 25 (47.17%) isolates were found to be positive for *P. aeruginosa* on the basis of growth characteristics on culture plates and biochemical properties and PCR. Among the 25 positive isolates of *P. aeruginosa* 3 were found from different instruments, 15 were found from floors and 7 were found from drain samples (Figure-1).

Proportion of samples positive for *S. aureus*

Among the 100 samples, 40 (51.28%) isolates were found to be positive for *S. aureus* on the basis of growth characteristics on culture plates and biochemical properties and PCR. Among the 40 positive isolates of *S. aureus* 8 were found from different instruments, 14 were found from floors and 18 were found from drain samples (Figure-1).

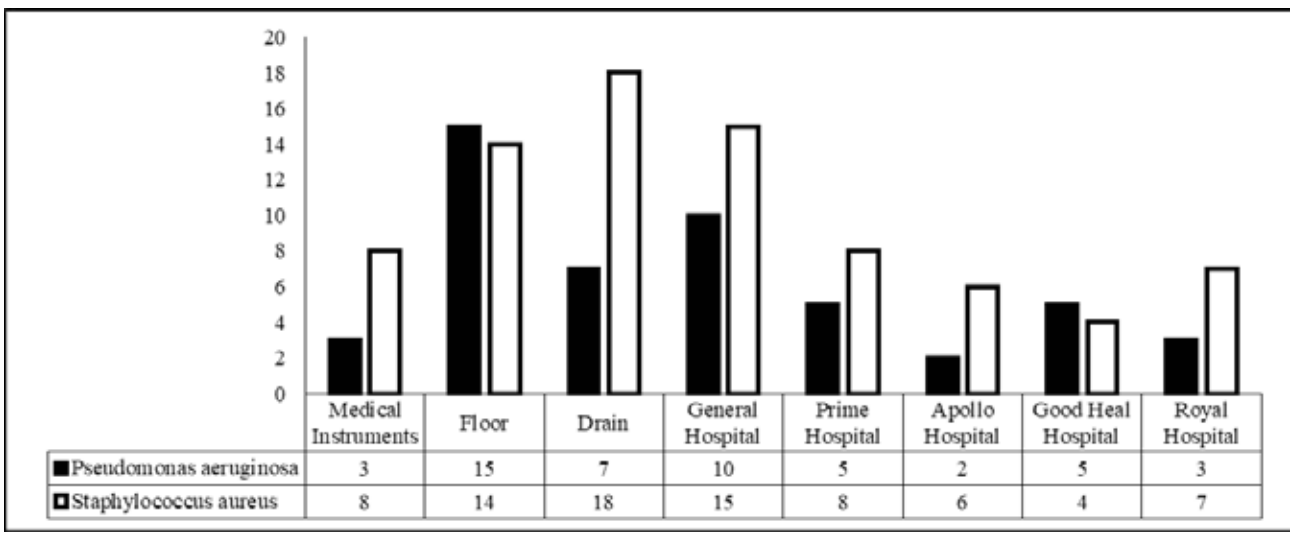

Figure 1: Proportion of samples positive for *P. aeruginosa* and *S. aureus*

Results of antibiotic sensitivity test of *P. aeruginosa* and *S. aureus*

After performing cultural and biochemical tests all the 25 *P. aeruginosa* and 40 coagulase positive *S. aureus* isolates were subjected to antimicrobial resistance profile assessment for phenotypic investigation of MDR (multidrug resistant). MDR is identified by assessing zone of inhibitions with different drugs. Figure 2 shows the multi-drug resistance pattern of all the 25 *P. aeruginosa* isolates. It is evident from the figure that all the isolates were more or less multi-drug resistant. All the isolates are resistant to Ceflazimide and Ceftriaone. Maximum sensitivity were found to Imipenem and Netilmicin. Figure 3 shows the multi-drug resistance pattern of coagulase positive *S. aureus*. It is clear from the figure that all the isolates were also more or less multi-drug resistant among them 25 isolates (62.5%) were identified as MRSA phenotypically. MRSA is identified by assessing zone of inhibitions with oxacillin< 10 mm and/or cefoxitin< 21 mm (CLSI. 2007). For this reason two types of discs containing Oxacillin or Methicillin (1 or 5mcg) and Cefoxitin (30mcg) were used for confirmation of MRSA. All other remaining isolates were intermediate multidrug resistant that were resistant against 4 to 7 antibiotics.

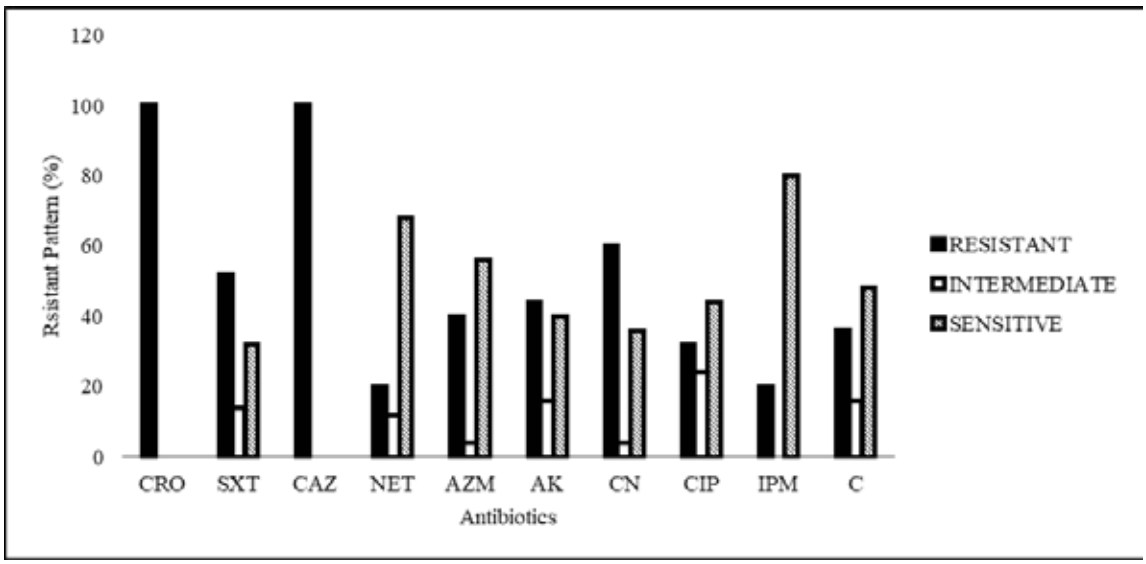

Figure 2: Bar Chart showing different antibiotic resistant pattern against *P. aeruginosa*

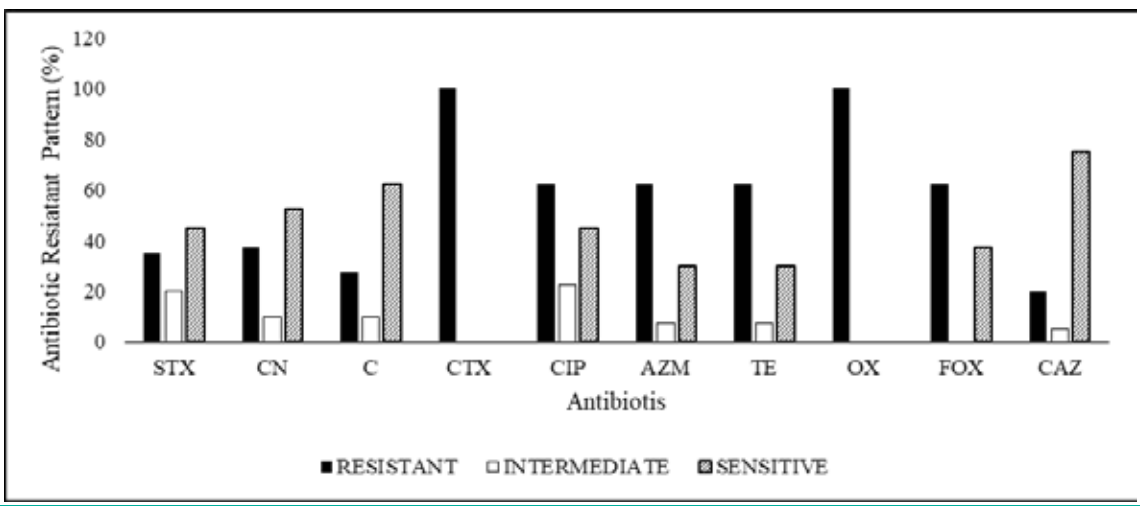

Figure 3: Bar Chart showing different antibiotic resistant pattern against *S. aureus*

Correlation of susceptibility profiles of the 25 isolates of *P. aeruginosa* against disinfectants and antibiotics

All the 25 isolates of *P. aeruginosa* are multidrug resistant where only three isolates (no. 1, 6 and 18) are highly resistant that resist 7 antibiotics. Antibiotic sensitivity pattern among all the isolates of *P. aeruginosa* was very different from each other. On the other hand all the 25 isolates showed very good sensitivity against the used concentrations of savlon and herpic. Among them eleven isolates (no. 3, 5, 10, 11, 14, 15, 16, 19, 20 22 and 24) showed MIC at 0.5% savlon solution and nine isolates (no. 4, 5, 6, 7, 10, 15, 16, 18 and 23) showed MIC at 2% harpic solution. All other isolates of *P. aeruginosa* showed MIC up to 0.7% for savlon and up to 2.25% for harpic which mean that their MIC for these disinfectants are increasing gradually due to improper use. Among the three highly multidrug resistant isolates isolates no. 6 and 18 showed their MIC at 2% herpic solution. Moreover, the remaining isolates of *P. aeruginosa* that was not highly antibiotic resistant showed MIC for savlon at 0.6% to 0.7% solution and herpic at 2.25% solution. So by comparing the susceptibility profile of the isolates of *P. aeruginosa* against disinfectants and antibiotics it can be said that antibiotic resistant and disinfectant resistant do not depend on each other or they are not interlinked (Figure-4).

Correlation of susceptibility profiles of the 40 isolates of *S. aureus* against disinfectants and antibiotics

All the 40 isolates of *S. aureus* are multidrug resistant where 10 isolates (no. 2, 11, 15, 16, 21, 22, 30, 32, 33 and 35) were found highly multidrug resistant that resist 7 antibiotics and 25 isolates (62.5%) were identified as MRSA phenotypically. On the other hand all the 40 isolates showed very good sensitivity against the used concentrations of savlon and herpic. Among them thirteen isolates (no. 1, 8, 9, 11, 14,21, 22, 24, 25, 30, 34, 36 and 39) showed MIC at 0.5% savlon solution and four isolates (no. 7, 16, 23 and 31) showed MIC at 1.75% herpic solution. All other isolates of *S. aureus* showed MIC up to 0.7% for savlon and 2.25% for herpic which mean that their MIC for these disinfectants are increasing gradually due to improper use. Among the 10 highly multidrug resistant isolates, isolates no. 16 showed MIC at 1.75% herpic solution and isolates no. 11, 21, 22, 30 showed MIC at 0.5% savlon solution. Moreover, the remaining isolates of *S. aureus* that are not highly antibiotic resistant showed MIC for savlon at 0.6% to 0.7% solution and herpic at 2% to 2.25% solution. So by comparing the susceptibility profile of the isolates of *S. aureus* against disinfectants and antibiotics it can be said that antibiotic resistant and disinfectant resistant do not depend on each other or they are not interlinked (Figure 5 and Figure 6).

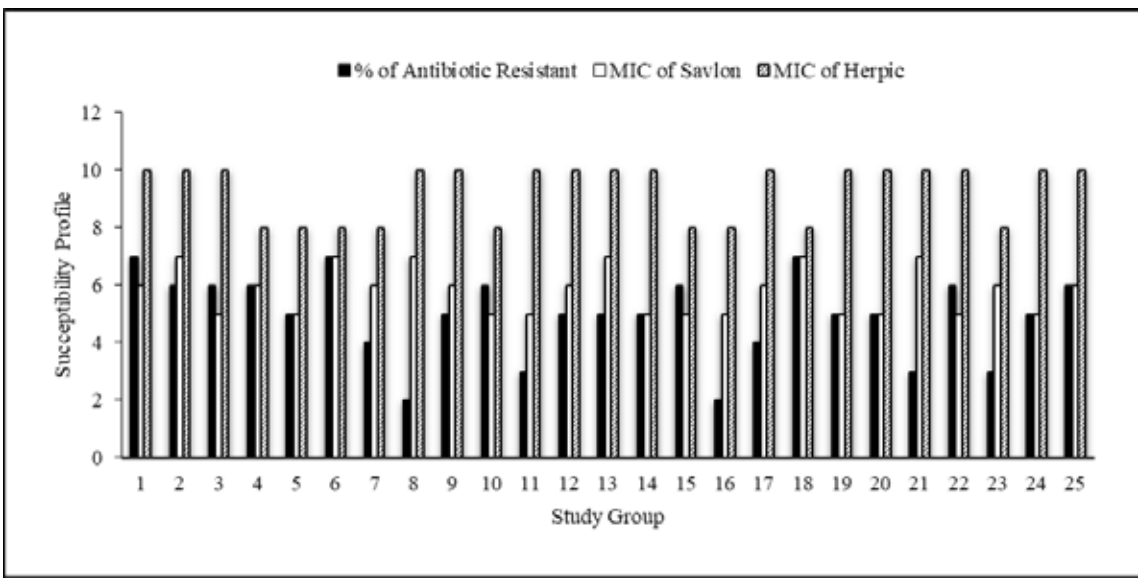

Figure 4: Susceptibility profiles of the 25 isolates of *P. aeruginosa* against disinfectants and antibiotics

Here, for savlon 1 unit is equal 10, for herpic 1 unit is equal 4, for antibiotics 10 units is equal 1.

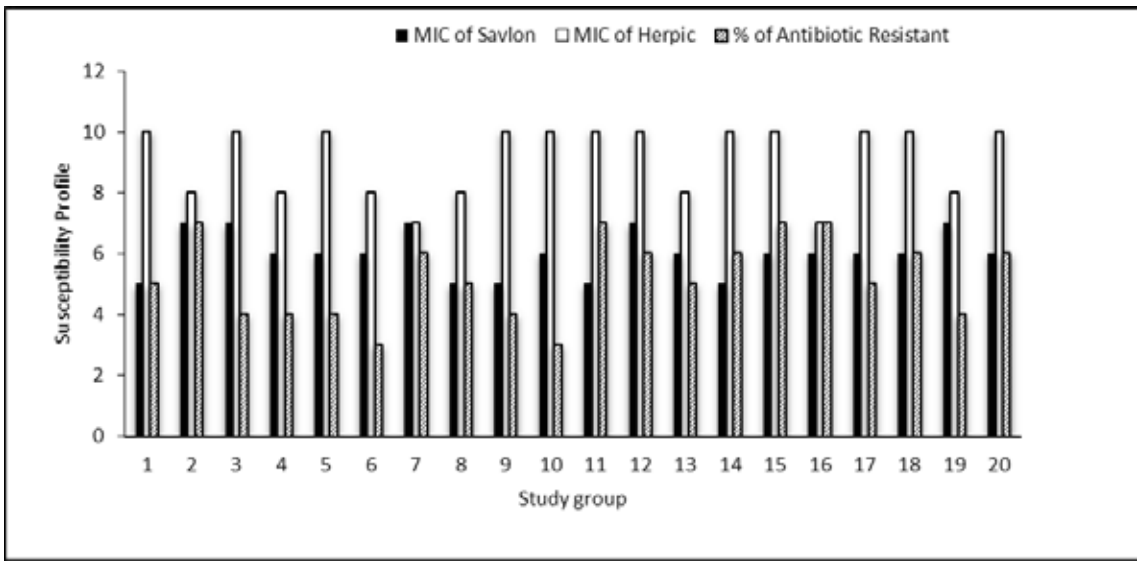

Figure 5: Susceptibility profiles of the first 20 isolates of *S. aureus* against disinfectants and antibiotics

Here, for savlon 1 unit is equal 10, for herpic 1 unit is equal 4, for antibiotics 10 units is equal 1.

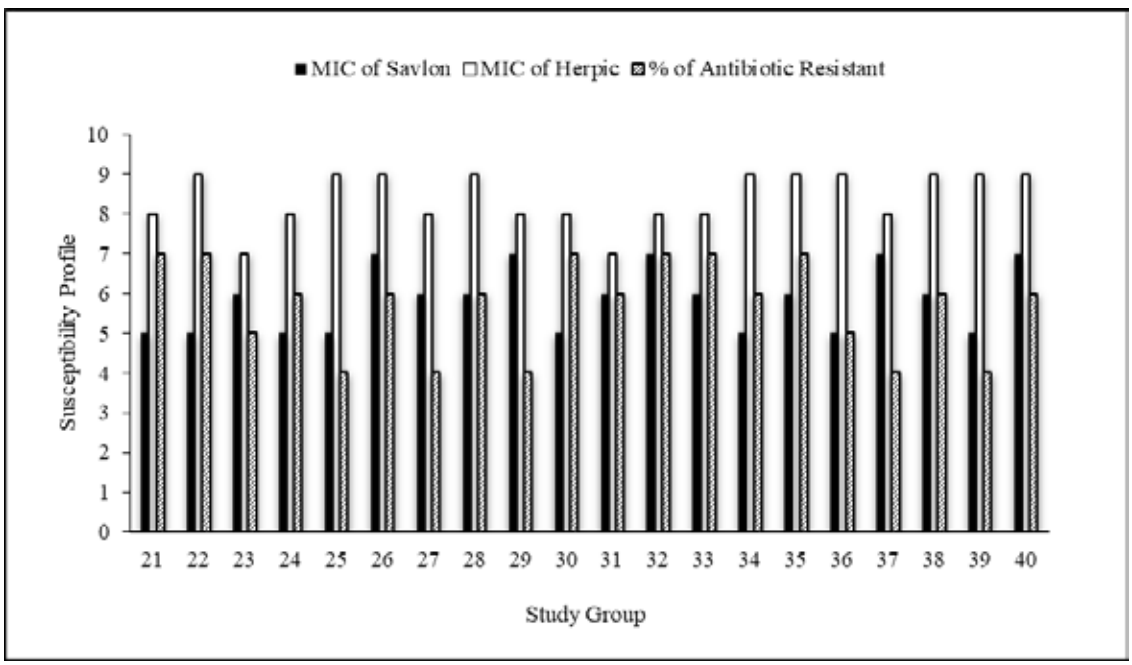

Figure 6: Susceptibility profiles of the last 20 isolates of *S. aureus* against disinfectants and antibiotics

Here, for savlon 1 unit is equal 10, for herpic 1 unit is equal 4, for antibiotics 10 units is equal 1.

Discussion

In ordinary health center exercise, disinfecting the floors and walls may not be essential. Through cleansing of floors and walls may be enough in less important regions, despite the fact that a few research endorse using a disinfectant in floor cleansing (31,32). Although cleaning may additionally dispose of a wide variety of bacteria but the microorganisms surviving

after the cleaning soon begin to develop, and might pass to contaminate other safe areas. It is extra essential to disinfect the "close to affected person" hand touch regions which might be one of the most crucial ways of transmission of infectious agents that is related to the hands of workers (32). So the use of biocides in vital and excessive-hazard areas as burn gadgets and Intensive Care Units (ICUs) is justified, (31-33) where there is a possibility of the presence of drug resistant pathogens in the environment, for example MRSA, Acinetobacter species and *P. aeruginosa* (34). On many events, at the same time as investigating outbreaks as a result of MRSA, we've done gross infection of the floors, utensils (beds, trays, equipment in Operation Theater), and drains associated with these hospitals. It is evident that 27.3% of the environmental surfaces like floors and walls in ICUs and emergency units are confirmed with Staphylococcus aureus contamination among them 30% of these have been MRSA (34). Perfect disinfection in the surfaces of operation theaters is critical (34). Previous studies emphasizes the fact that ordinary cleansing, hand washing or barrier nursing on my own were now not enough to provide protection from the outbreaks of MRSA, but having requirement for proper disinfection of the surroundings (35,36).

Different types of biocides are commercially available that undergo substantial trying out in particular conditions for getting release to the market. However, frequently, the goods and strategies may not be capable of competently disinfect gadgets whilst the surfaces had been infected with exceedingly resistant or uncommon microorganisms, or if there is a very heavy high load of microbes. This fact can be counted in addition complication due to air particle or other organic count number. When selecting a disinfectant for unique sanatorium use, it is able to be essential to recognize the predicted variety and the sorts of organisms possibly to be gift at the floor. Therefore the selection of disinfectants should be based on its capacity to kill microorganisms and prevention of their transmission (36). So the disinfectants of choice should contain wide antimicrobial activity, nonirritating, less poisonous, noncorrosive being less expensive (37).

In this study 'in-house concentrations' was used keeping in mind the safety of staffs of hospitals and the sterilization of different instruments and surfaces of these hospitals. In a previous study, clinical bacterial isolates of *Pseudomonas* spp. was found (38) which can tolerate in-house concentrations of disinfectants that differ from the result of our study with *P. aeruginosa* and *S. aureus*. More precisely, 25 isolates of *P. aeruginosa* and 40 isolates of *Staphylococcus aureus* were exposed to two disinfectants and the MIC of the disinfectants were found to be 0.5% to 0.7% (v/v) for savlon and 2% to 2.5% (v/v) for herpic except four isolates of *S. aureus* that showed MIC against herpic at 1.75%, higher than their in-use concentrations. No sign of co-resistant of disinfectant and antibiotics were found. Similar result was found from the previous study of Martróet al. (22) and Wisplinghoffet al. (23) who observed no clear evidence of resistant within the clinical isolates of *Acinetobacter* spp.

Russell et al. (39) observed that *P. stutzeri* developed resistance to chlorhexidine gluconate which is related with the development of resistance to polymyxin B, gentamicin, erythromycin and ampicillin. On the other hand, same results were found with MRSA and *P. aeruginosa* (40,25). Our results showed no clear correlations between the decreased susceptibility to savlon and herpic with the resistance to antibiotics among hospital isolates of *P. aeruginosa* and *S. aureus*.

In the general practices in the hospitals of Bangladesh, specific guideline for the use of disinfectants is not followed due to ignorance or negligence. The development of disinfectant reduced susceptible bacteria may be a result of it. But as the MIC of the used disinfectants are increasing, there may be a common molecular mechanism present in the bacteria for being disinfectant and multi drug resistant. If the mode of action of a disinfectant coincides with the mode of action of an antibiotic, in that case the co resistant may occur. Though it is a hypothetical statement, some previous studies reports that the antimicrobial effectiveness of numerous disinfectants have been remarkably reduced may be due to the inhibition in the presence of organic matter (41,42).

Conclusion

As several hospital isolates of *P. aeruginosa* and *S. aureus* are showing reduced susceptibility to disinfectants and all the isolates are multi drug resistant but it can be said in summery that disinfectants (savlon and herpic) can't be a cause for *P. aeruginosa* and *S. aureus* to become multidrug resistant because no clear correlation were observed after analyzing all the data when these disinfectants are improperly used.

Abbreviations

MSSA: Methicillin-Susceptive Staphylococcus aureus; ***PCR:*** Polymerase Chain Reaction; ***MIC:*** Minimum Inhibitory Concentration; ***DNA:*** Deoxy Ribonucleic Acid; ***TBE:*** Tris Borate ***EDTA; UV:*** Ultra violate; ***MDR:*** Multi-Drug Resistant; ***MRSA:*** Methicillin-Resistant Staphylococcus aureus.

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Disclosures regarding real or perceived conflicts of interest

No competing interests in this scientific work.

Contribution Categories	Name of Author
Development of study idea	T.I., O.S., S.S., M.H.
Methodological design of the study	T.I., O.S., S.S., M.H.
Data acquisition and processing	T.I., M.M.R., S.S.
Data analysis and interpretation	M.S.H., S.M., M.H.
Literature review	T.I., O.S., M.M.R.
Manuscript write-up	T.I., S.M., S.S., M.H.
Manuscript review and revision	M.M.R., M.H., M.H.

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