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PHENOTYPIC RESISTANCE OF NOSOCOMIAL BACTERIAL ISOLATES TO SOME ROUTINELY USED DISINFECTANTS

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ABSTRACT

The rise in biocide resistance among nosocomial isolates is a well-travelled narrative and Bioaerosols are recognized causes of Hospital acquired infections. This study verifies the presence of bioaerosols in hospital ward and evaluates the effectiveness of some biocides; Dettol® (Chloroxylenol), Izal® (Saponated cresol), and Jik® (Sodium hypochlorite), commonly used in the Specialist Hospital, Sokoto, Nigeria at their manufacturers recommended concentrations and a concentration slightly above it against nosocomial bacteria. Salmonella species (23.9%), *Staphylococcus aureus* (19.7%), *E. coli* (15.5%), *Enterobacter* species (12.7%), *Pseudomonas* species (11.3%) were the most frequently isolated bioaerosols. All the bacterial isolates were resistant to the three biocides tested in this study. The outcome of the study revealed Jik® as the least resisted biocide. Indiscriminate use of these biocides should be avoided if environmental contamination in the ward is to be effectively controlled.

KEYWORDS: Disinfectant, Biocides, Bioaerosols, Sokoto, Specialist Hospital, Resistance. Nosocomial infections.

INTRODUCTION

Disinfectants in general refers chemical agents employed to disinfect lifeless articles.[1] The use of chemical disinfectants in hindering the growth of pathogenic microorganisms is known as disinfection.[1] These chemical agents are used to abate or destroy pathogenic microorganisms in or on materials with a resolve to make them safe. Disinfection is however not a flat-out term, as this submits that some organisms may not be affected yet the likelihood of sterilization coming about because of disinfection cannot be discounted.[2]

Proper disinfection practice is an absolute necessity for control of nosocomial infections, as negligence can bring about increase nosocomial infection rate, consequently prompting rise in cost, morbidity and mortality.[3] In hospital environment, disinfection is principally accomplished either by surface disinfection or immersing the dirty objects in the disinfectant solution.[4] Disinfectants may similarly be utilized to chemically treat hazardous waste, particularly the microbiological wastes and disposable plastics. Factors like temperature, contact period, pH and concentration, bioburden, organic soil and hardness of water utilized for dilution may the affect rate of disinfection.[5]

Bio-aerosols are airborne particles that are living (bacteria, viruses and fungi), originate from living organisms or manufactured surfaces. Each source gives rise to an entirely unique assemblage of bio-aerosols.[6] The microbial diversity in hospital indoor air is highly affected by the number of indwellers, their activity and the ventilation. Sweeping of floors and changing of bed linens also can cause suspension of bio-aerosols in air.[6,7,8] Bio-aerosols can be spread either at long distances beyond the patient room environment, or within short distances. Bio-aerosols are a major source of nosocomial infection in immunocompromised patients.[6]

Whether or not a biocide resistance occurs depends to a large extent on the concentration of the active molecule in the product and how it is used.[8] In most of the early cases (1950s), resistance developed because the biocides were used or stored improperly, so that the concentration of the biocide in the product was too low to be effective.[9,10] Since then, reports of resistance to biocides and to all known preservatives has increased.[10]

Some biocides currently used in hospitals were found to be ineffective against biofilms producing bacteria attached to surfaces, and this may have an important role in the transmission of nosocomial infections.[11]

Bacteria may become resistant by; Modification cell envelope structure to reduce the influx biocides (e.g. biofilms producers), activating an efflux pump system that "pumps out" toxic compounds, activation of enzymes that cause degradation by causing chemical changes in biocides, by acquiring resistance genes.[12] Bacteria secretes certain "signal" molecules that other bacteria can detect so the whole colony activates specific genetic cascades involved in the formation of biofilms and thus develop resistance.[13]

Appropriate disinfection and sterilization procedures are an unquestionable requirement for control of hospital acquired infection (HAIS), as neglect can bring about numerous Hais in this manner prompting increased cost.[13]

This study evaluates the effectiveness of some disinfectants commonly used in the hospital at their manufacturers recommended concentrations and a concentration slightly above it against some bacteria of nosocomial origin.

METHODS

Sample Collection Center

The Specialist Hospital Sokoto (SHS) was chosen as the sample collection center. SHS is a referral hospital equipped with about 300 beds with modern equipment and facilities saddled with providing health care services. The hospital is patronized by people from within Sokoto state and its surrounding localities.

Ethical consideration

Ethical clearance was obtained from the hospital management before samples were taken.

Bacteriological Study of the Wards

Plates containing Nutrient agar, MacConkey and Blood agar were exposed in the wards twice a week for an hour throughout the duration of the study. Thereafter, the plates were covered and taken to the laboratory for incubation at 37^{o}C for 24hrs. The morphological characteristics of the emergent colonies were examined and isolated in pure culture, then sub-cultured in nutrient agar slant and kept in refrigerator for further characterization.

Characterization of Bacteria Isolates

The isolated bacteria were characterized based on their cultural, morphological and biochemical reactions as described by.[14] and.[15]

Determination of Susceptibility of Isolates to Selected Disinfectants

The most commonly used disinfectants in Specialist Hospital Sokoto were Dettol® (chloroxylenol), Izal® and Jik® The test organisms were prepared and compared with 0.5 McFarland turbidity standard using a U.V spectrophotometer.

Preparation of the test concentrations of disinfectant formulations

Two test dilutions each of the three disinfectant formulations were prepared in such a way that the concentrations were in compliance with and slightly above the manufacturers recommended concentrations. The test concentrations were obtained using the dilution factor formula.

Using sterile 5ml syringe, two volumes, 3ml and 6ml of Dettol® was obtained and diluted in 97ml and 94ml of distilled water in a conical flask respectively. The resultant percentage concentration was 3%v/v and 6%v/v respectively. Similar procedure was used for the other disinfectants; 0.5ml and 1ml of Izal® and 2.5ml and 5ml Jik®, to give percentage concentration of 0.5%v/v and 1%v/v and 2.5%v/v and 5%v/v respectively.

Antimicrobial Screening of the Disinfectant

Using agar diffusion method, two dilutions each, of the three test disinfectant formulations were used. Twenty milliliters of melted and cooled Mueller-Hinton agar were poured into each Petri dish and allowed to set. The test organisms were spread aseptically on the surface of the agar using a sterile swab stick and allowed to dry for about an hour. Five wells were bored into the agar plates using 8mm cork borer. Using a micropipette, the first three wells were filled with two drops of the manufacturer's dilutions for the three disinfectant formulations (3%v/v Dettol®, 0.5%v/v Izal® and 2.5%v/v Jik®) and 10µg of gentamicin were introduced into the fourth well as positive control while sterile distilled water was introduced into the fifth well as negative control.[16]

This whole process was done in duplicates. The plates were then incubated at 37^{o}C for 24 hrs. The corresponding zones, of growth inhibition were recorded.[16]

Statistical Analysis: Microsoft excel 2016 was used after tabulation of bacteria isolate abundance and zone of inhibition measurements to carry out descriptive statistics.

RESULTS

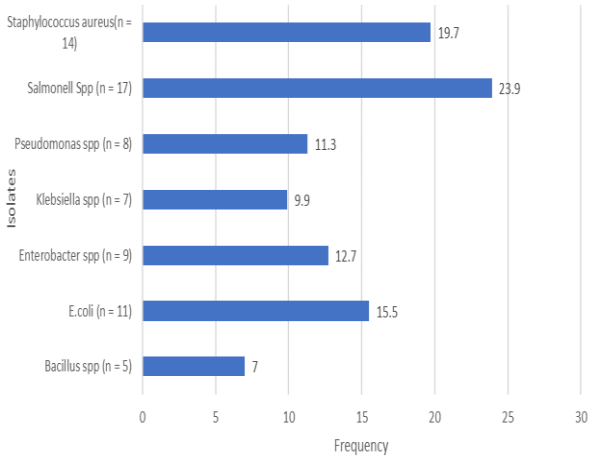

Figure 1: Relative Abundance of bacteria collected from ward air.

Different bacteria species belonging mostly to the enterobacteriaceae families and *Klebsiella* spp. were isolated from the indoor air of surgical ward of the Obstetrics and Gynaecology Department of the specialist hospital Sokoto (Fig,1). The most frequently isolated organisms were *Salmonella* spp. and *Escherichia coli*.

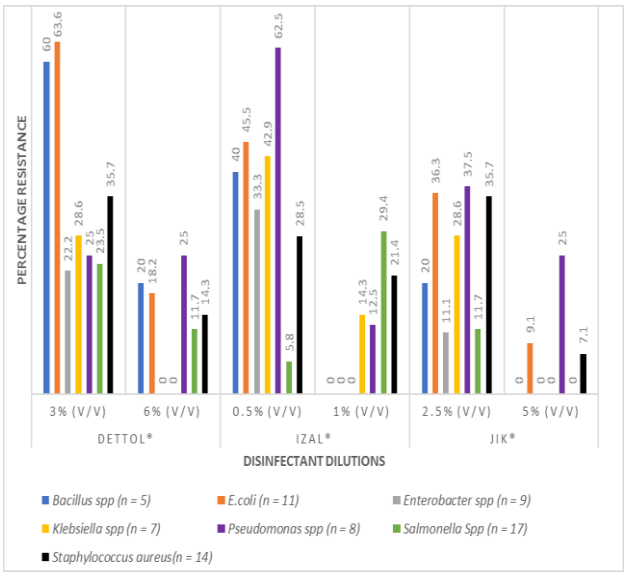

Figure 2: Percentage resistance profiles of bacterial isolates from the indoor air of O & G ward to the test disinfectants.

Figure 2, shows that few of the bacteria isolates from the O & G ward were resistant to some of the disinfectant at manufacturers prescribed concentration as used in this study. *Escherichia coli*, *Pseudomonas* spp. and *Klebsiella* spp. were resistant to 3%v/v Dettol® and 0.5%v/v Izal® but susceptible to Dettol® 6%v/v and Izal® 1%v/v.

DISCUSSION

Examination of the indoor air of the ward revealed potentially pathogenic organisms present in the atmosphere (bio-aerosols). In this study, 71 bacterial isolates of 7 bacterial species were identified from the indoor air using settling plate method. *Salmonella* species (23.9%), *Staphylococcus aureus* (19.7%), *E. coli* (15.5%), *Enterobacter* species (12.7%), *Pseudomonas* species (11.3%), *Klebsiella* species (9.9%) and *Bacillus* species (7%) were the bacterial isolates obtained in this study. Most of these isolates are known to be pathogenic or potentially pathogenic. They are opportunistic, notorious for causing several nosocomial infections, such as skin, lungs, eye infections, digestive disorders and endocarditis. The distribution of the bacterial isolated from indoor air of the Obstetrics and Gynecology ward is similar to those found by.[17]

Salmonella species were the most frequently isolated organisms in this study. The presence of *Salmonella* isolates among bioaerosols obtained can be cited to the statement that it may have been dispersed by winged insects (houseflies and cockroaches) as they fly over the exposed plates.[18,19] These insects which may have had previous contact with fecal material are known potential vectors for bacterial pathogens. Cockroaches and houseflies are found around hospitals, wards and sick-rooms. In addition, several published papers have recognized likelihood of flying insects in transmission of nosocomial infections.[20,21,22,23,24]

The statement that *Staphylococcus aureus* is a major cause of nosocomial infection is a well-travelled narrative.[25] Accordingly, the relative abundance of *Staphylococcus aureus* realized in this study is not unexpected.

The resistance of the bacterial isolates to three disinfectant frequently used by the hospital; Dettol®(Chloroxylenol), Izal® (Phenol), and Jik® (Sodium hypochlorite) were assessed with two concentrations, the product manufacturers recommended concentration and one above the aforementioned.

At the manufacturers concentration, the isolates were resistant to all three disinfectants. The resistance to biocides used here can be categorized into three resistance levels; Dettol (the most resisted), Izal (the second category) and Jik (the least resisted). The observed level of resistance in this study may be due to the development of resistance by naturally selected isolates that emerged from previous exposure to indiscriminate use of biocides.[26] Resistance may also occur as result of spoilage or improper storage of biocides.[27]

The difference in resistance is probably due to the intended use of the biocide (as external disinfectant or as antiseptics), as this will determine the amount of active ingredients in the commercial products.[28,29]

At concentration, slightly above the manufacturer

recommendation, a decrease in percentage resistance was observed. At this concentration, some isolates (*Enterobacter* sp, *Klebsiella* sp, *Bacillus* sp, *E. coli* and *Salmonella* sp) lost their resistance.

Another reason that has been advanced to explained the wide spread resistance to biocides is the phenomenon of cross resistance.[29] Exposing bacteria to some antibiotics can activate the genes responsible for resistance against both biocides and antibiotics by activating genetic controls that are involved in triggering resistance mechanisms that alter both biocide and antibiotic activity. If the loci of genes conferring resistance to both are found near to the other, the two sets of gene may be transferred together.[30]

Staphylococcus aureus and *Pseudomonas* sp resistance persisted relatively in all the test concentration. This may be explained by the virulent nature of these two organisms. *Staphylococcus aureus* and *Pseudomonas* sp are biofilm produces.[31] Biofilms are encased in polysaccharide layers that lessen the diffusion of antimicrobials. Compared to free cells, bacteria in biofilms are more concentrated, grow more slowly and are in a different physiological state; and all this could affect their susceptibility to biocides.[32]

CONCLUSION

The outcome of this study has revealed that the indoor air of the hospital ward comprises biocide resistant nosocomial bacterial pathogens. Most of the isolated bacteria species circulating in the wards thrived above biocide manufacturers stated in–use dilutions. A lesser extent of bacterial resistant was observed at higher disinfectant- concentrations. The sodium hypochlorite-containing disinfectant (Jik®) was least resisted by the bacteria isolates. The high resistance highlighted in this study may be sufficient to warrant the use of these agents at higher concentration than recommended. Indiscriminate use of these biocides should also be avoided if environmental contamination in the ward is to be effectively controlled.

Limitation

1. Phenolic coefficient of the disinfectants were not determined.
2. The purpose of bio-aerosol sampling in this work was to verify their presence and not to quantify their presence.

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