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Prevalence and multidrug resistance profiles of several bacterial pathogens isolated from hospital inanimate surfaces in Faisalabad, Pakistan

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Abstract

Hospital environment and inanimate surfaces are considered as potential sources of opportunistic and nosocomial pathogens. Indirect transmission of microbes from the hospital surfaces has a major role in hospital acquired nosocomial infections and their colonization. The present study was designed to investigate the prevalence of major pathogenic bacteria isolated from hospital inanimate surfaces. Random swab samples were taken from inanimate surfaces and apparatus used in daily treatment of patients from the major hospitals in district of Faisalabad, Pakistan. These swab samples were cultivated on suitable culture media including; nutrient agar, MacConkey's agar and blood agar to isolate the bacterial pathogens. Identification of the bacterial cultures was carried out by observing the cultural and macroscopic characteristics including Gram staining. Further verification of these bacterial cultures was carried out using appropriate biochemical assays. The biochemical assays were performed for characterization of *Staphylococcus aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*. These pure cultures were then tested for resistance to different antibiotics using the Kirby–Bauer method. The prevalence of Gram positive bacteria (60.83%) was more than that of Gram negative bacteria (24.13%). The results of antibiotics susceptibility tests were analyzed statistically.

Keywords: Bacterial pathogens, Nosocomial infection; Inanimate surfaces; Kirby-Bauer method

1. Introduction

Environmental surface contamination serves as a perspective reservoir for epidemic and endemic pathogens to cause different diseases in hospitalized or immune-compromised patients (Saka *et al*., 2016). Hospital inanimate surfaces include; tray tables, bed rails and supply carts which are considered as the most significant mode of transmission of Healthcare Associated Infections (HAIs). The rate of pathogens transmission in hospital rooms that are occupied by infected patients is enhanced to 40% as compared to those rooms where the previous occupant was not infected. Jinadatha *et al*., (2017) reported that the risk of bacterial contamination is directly related to the contaminated environment, when there is no chance of contact between two infected patients. Harris *et al*., (2006) added that factors that influence the acquisition of a nosocomial antibiotic-resistant bacterial infection include poor epidemiological techniques such as; crowding, hand washing, and lack of sufficient scientific knowledge about the proper use of antibiotics. These antibiotics cause resistance against the bacterial infections, and thus increase the rate of colonization of microorganisms of the hospital inanimate environment.

Multidrug resistant (MDR) microbial pathogens show resistance against various antimicrobial drugs. The reasons behind induction of MDR in microbial pathogens include irrational use of antibiotics, transfer of genes/ plasmids, and inappropriate guidelines about the use of these antibiotics. Espinal *et al*., (2012) previously documented that some bacteria like *Acinetobacter baumannii* have the ability to form biofilms. Biofilm contains active polymeric substances which are beneficial for the bacteria and enable them to resist the various antibacterial agents. Catano *et al*., (2012) added that among the patients suffering from bacterial infections, antibiotic resistance simply causes the long period of hospital stay in addition to mortality and morbidity potential of the disease. The aim of this study is to check the prevalence of multi drug resistant pathogenic bacteria in the hospital environment and inanimate surfaces which cause various nosocomial infections.

2. Material and methods

The present study was performed in the laboratory of Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan, and was conducted in compliance with the Institutional Biosafety Committee (IBC) of the University of Agriculture.

2.1. Collection of samples, isolation of bacteria from contaminated hospital surfaces

A total number of 120 swab samples were taken from the inanimate surfaces of 4 different hospitals including; chairs, tables, medicine trays, curtains, computers, door handles, windows, doctor phones, side tables, and from the patients beds that are considered as the main sources of bacterial contamination. Swab samples were transferred immediately and under aseptic conditions to the microbiology laboratory. The swabs were then streaked on the surface of Nutrient agar, Blood agar, MacConkey's agar, Mannitol Salt agar and Cetrimide agar media, and then incubated at 37°C for 24-48 h to check for bacterial growth.

2.2. Identification of the bacterial isolates

After incubation, the recovered bacterial isolates were identified using Gram staining, and biochemical tests including; Indole Test, Oxidase Test, Methyl red Test, Catalase Test, Coagulase Test, Triple Sugar Iron test, Salt tolerance Test, Urease Test, Novobiocin Test, and Voges-Proskauer Test (VP), according to Cappuccino and Sherman, (2005).

2.3. Antibacterial susceptibility test

Pure cultures of six isolates were subjected to antibiotic resistance testing using Kirby Bauer disc diffusion method. Bacterial suspension of 24 h old culture was spread on Muller Hinton (MH) agar plates. The antibiotics disks were placed on the surface of the plates individually, and then incubated at 37°C for 24 h (Rutala *et al*., 1997). Various discs including; Ceftriaxone, Cefoxime, Amikacin, Ciprofloxacin, Nitrofurantoin, Norfloxacin, Ampicillin, Gentamicin, Amoxicillin, and Amoxicillin with Clavulanic acid, were used in reference to Humphries *et al*., (2018). This procedure was repeated twice with three replicates for each isolate against each antibiotic. The Clinical laboratory standard guidelines were used to determine the resistance pattern of the different bacterial isolates against the different antibiotics according to Humphries *et al*., (2018). The bacterial isolates that showed resistance ≥ 3 antibiotics were considered as multidrug resistant (MDR) pathogenic bacteria.

2.4. Statistical analysis

Results of the antibiotic susceptibility test were subjected to statistical analysis. Bi-variant charts chi-square method has been used that briefly showed the relationship between the isolated bacteria and the antibiotic discs. The P-value presented the non-significant (P> 0.05) and the significant (P< 0.05) difference in the prevalence of the isolated bacteria according to Ilstrup, (1990).

3. Results

3.1. The isolation, identification of bacteria from in-animate hospital surfaces

About six bacterial isolates are recovered on the isolation media. These isolates are identified according to the morphological, microscopic and biochemical characteristics as; *Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *K. pneumonia*, *P. aeruginosa* and *E. coli*. The prevalence rate of the Gram-positive bacteria is 60.83% (73/ 120), whereas that of the Gram-negative bacteria is 24.13% (29/ 120). The remaining 16% positive samples included miscellaneous bacterial and fungal contamination. The overall prevalence of the Gram positive bacteria was high compared to the Gram negative bacteria.

The overall Gram positive bacteria (60.83%) are comprised of *Staph. aureus* (40%), *Staph. epidermidis* (12.5%), and *Staph. saprophyticus* (8.33%). On the other hand, the Gram negative bacteria (24.13%) are comprised of *E. coli* (10.83%), *K. pneumonia* (7.5%) and *P. aeruginosa* (5.83%) as clear in Fig. (1).

3.2. Antibacterial susceptibility test

The antibiotic susceptibility profile of *Staph. epidermidis* is observed as a clear zone formed around the antibiotic discs. This bacterium showed appreciable resistance patterns against several antibiotics including; Gentamicin (90%), Ceftriaxone (80%), Cefoxime (100%) and Ampicillina (100%) (Fig. 2a).

Similarly, *Staph. saprophyticus* (Fig. 2b) has also shown different sensitivity patterns against the different antibiotics such as; Nitrofurantoin (90%), and Amoxcillin (60%). The recorded antibiotics sensitivity behavior of *Staph. aureus* isolate include; Amoxcillin with clavulanic acid (89.59%), Cefoxime and Norfloxacin (77.09%) and Amikacin (72.84%) (Fig. 2c).

On the other hand, the Gram negative bacteria such as *E. coli* exhibited noticeable resistance against the different antibiotics including; Cefoxime (92.3%), Norfloxacin (92%) and Ceftriaxone (76%) (Fig. 2d). The antibiotic resistance pattern depicted by *K. pneumonia* has been observed in (Fig. 2e), which demonstrates appreciable resistance against Amoxicillin (85.71%), and Gentamicin (60%). Conversely, it showed significant sensitive behavior against several antibiotics mainly; Amikacin (100%), Ampicillin (85%), Nitrofurantoin (85.61%), and Ceftazidime (71.42%).

Finally, the antibiotic susceptibility of *P. aeruginosa* has been described by clear zone formation. This isolate expressed high resistance to several antibiotics such as; Amoxicillin (88.88%), Ceftriaxone (66.66%), and Amikacin (60%). However, *P. aeruginosa* showed sensitivity to; Ciprofloaxacin (88.88%), Nitrofurantoin (77.77%), and Cefoxime (66.66%) as clear in Fig. 2(f).

3.3. Statistical Analysis

The statistical analysis of the sensitivity of the bacterial isolates to the different antibiotics including; Amoxicilin, Ciprofloxacin and Ampicillin does not show significant difference as P> 0.05. Moreover, the antibiotics Gentamycin, Amoxicillin, Nitrofurantoin, Ceftriaxone, Cefoxime, Ceftazidime, Amikacin and Norfloxacin do not express significant inhibitory effects on the isolated bacteria as P< 0.05.

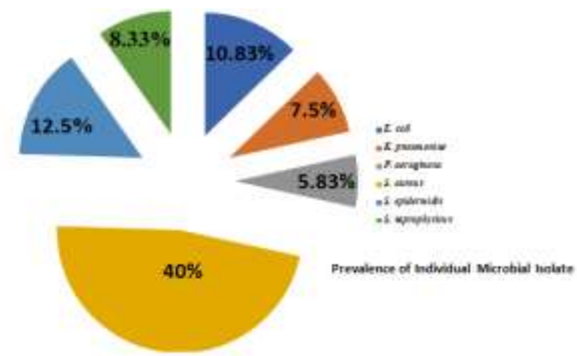


Fig. 1: Prevalence of the six bacterial spp. isolated from different inanimate surfaces on Nutrient agar, Blood agar, MacConkey's agar, Mannitol Salt agar and Cetrimide agar media.

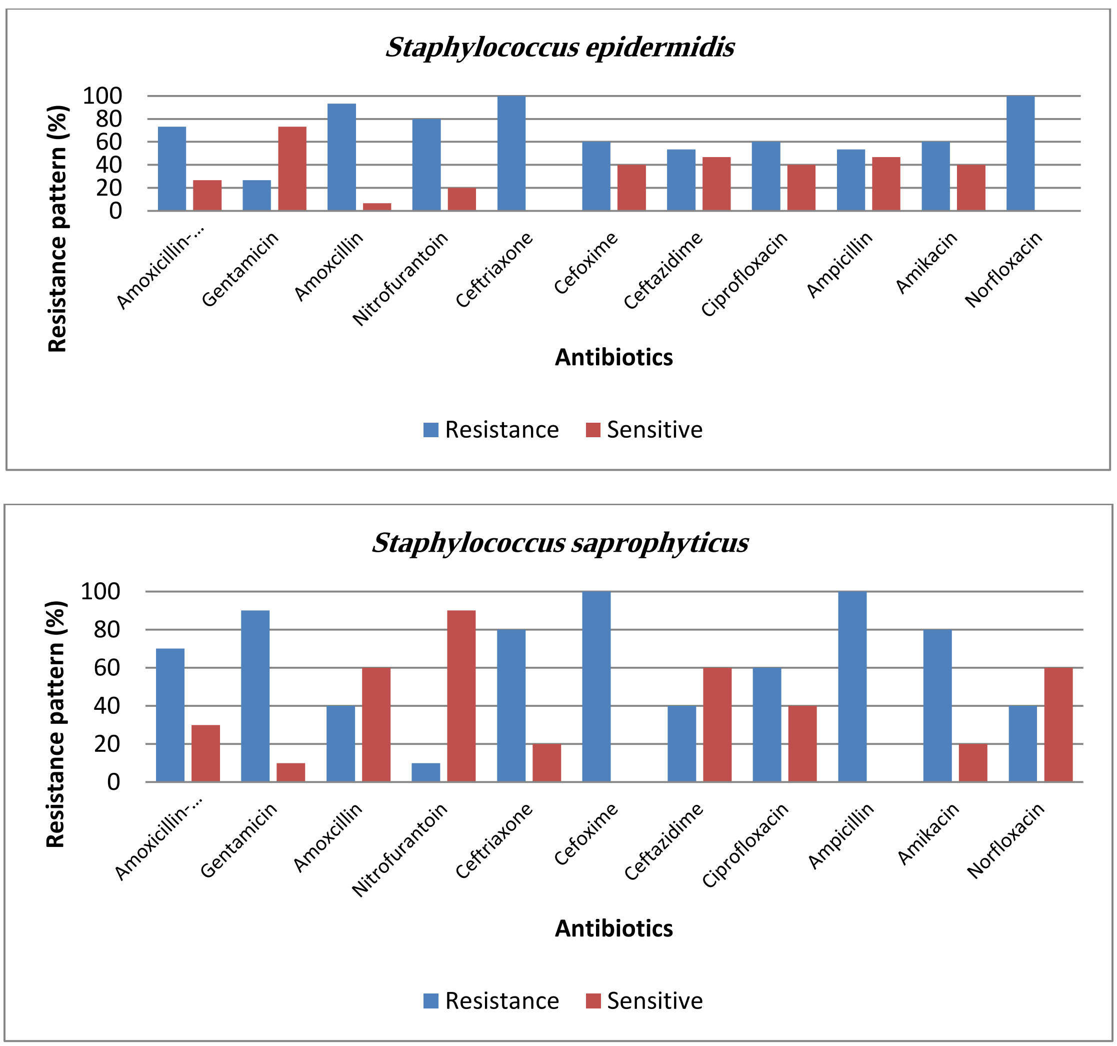
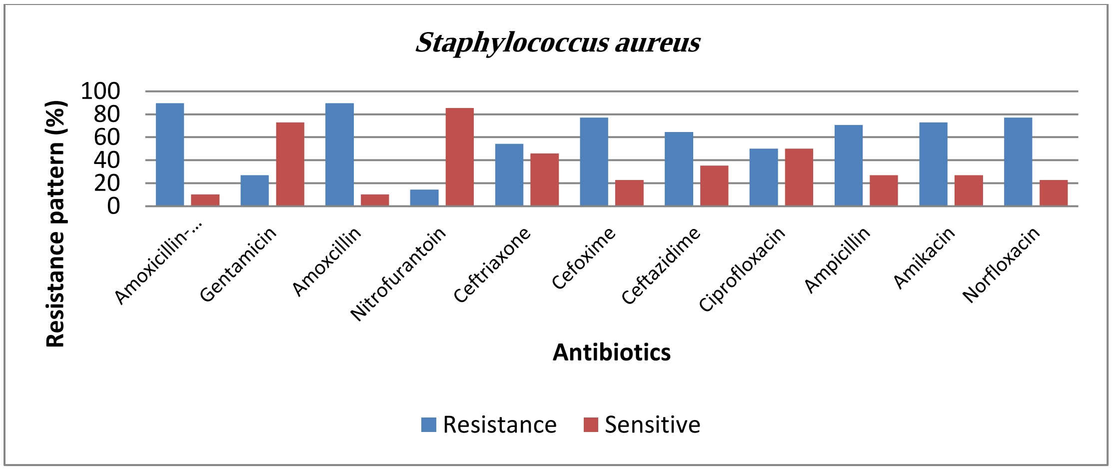


Fig. 2: (a) Resistance patterns recorded by *Staph. epidermidis* against different antibiotics, **(b)** Resistance patterns of different antibiotics against *Staph. saprophyticus*, using the Disc diffusion method.



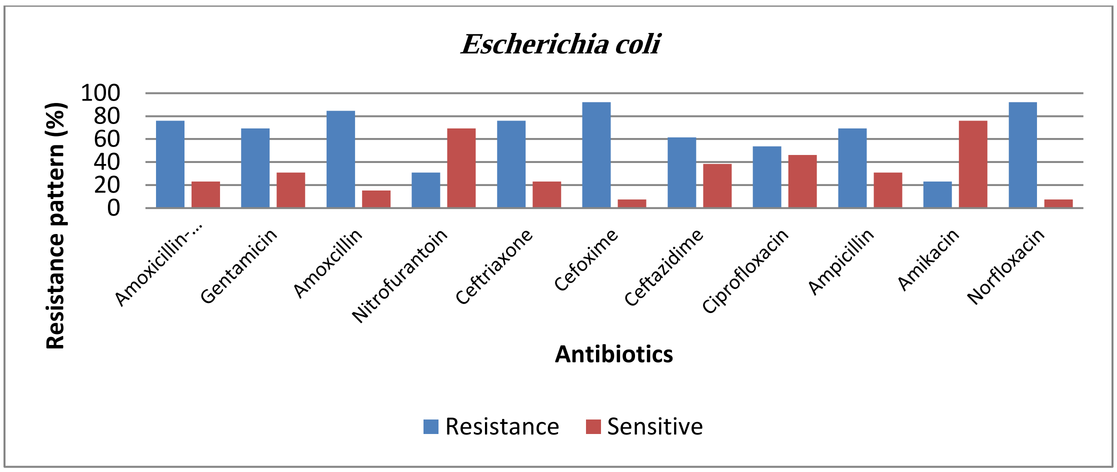
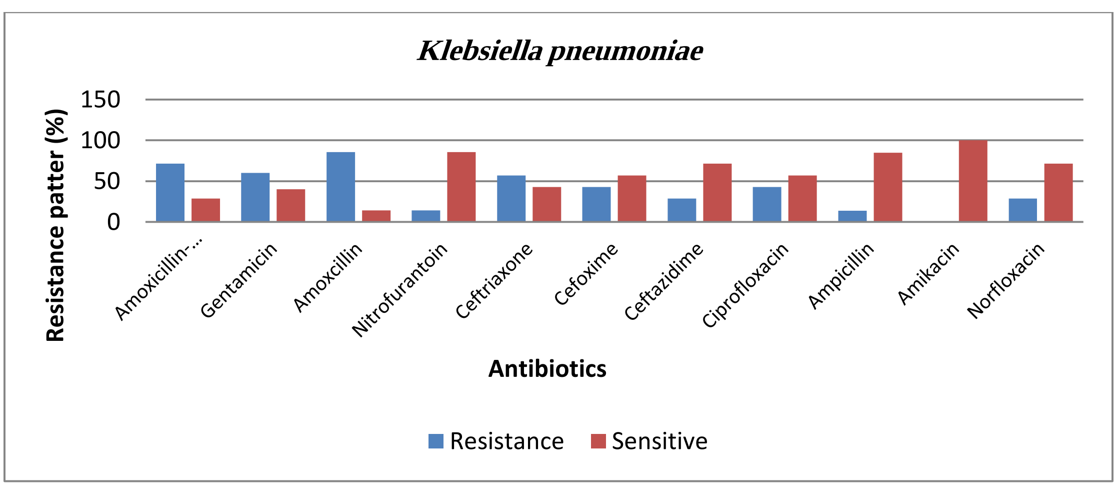


Fig. 2: **(c)** Resistance patterns against the different antibiotics recorded by *Staph. aureus*, **(d)** Resistance patterns of different antibiotics against *E. coli*, using the Disc diffusion method.



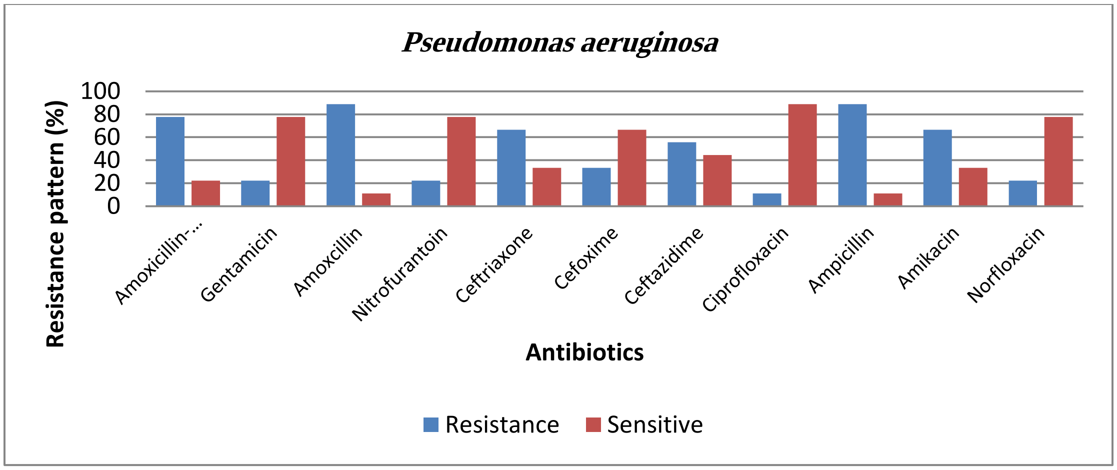


Fig. 2: **(e)** Resistance profile shown by *K. pneumonia* against the different antibiotics, **(f)** Resistance profile of *P. aeruginosa* against the different antibiotics, using the Disc diffusion method.

4. Discussion

The human touch of hospital inanimate surfaces is considered as a potential source of nosocomial infections. Current cleaning and disinfection of these surfaces are not successful to eradicate the bacterial infections completely but somehow may minimize their risk. Han *et al*., (2015) reported that methicillin resistant bacterial spp. such as *Staph. aureus*, *Acinetobacter baumannii*, *Clostridium difficile*, and vancomycin resistant Enterococci are the most significant bacterial pathogens that were isolated from infected patients and survived for longer period on inanimate surfaces. The highest prevalence of all those isolated bacteria is recorded by *Staph. aureus* (40%). This may be due to the availability of suitable conditions in the hospitals that may prolong the consistency of this bacterium. Accordingly, *Staph. aureus* may be the potential source of bacterial contamination that causes human nosocomial infections (Adam *et al*., 2019).

The present study reported that the Healthcare workers (HCW's) are reducing the bacterial contamination in the hospitals environment by proper hand washing before and after attending the patients. By doing such practice the prevalence of the bacterial pathogens on hospital surfaces will be reduced to 50%. The reduction of biofilm formation by bacteria takes place only when dirtiness is removed routinely from the hospital surfaces. In endemic situations, the prevalence of MRSA and VRE is enhanced, but during outbreaks *P. aeruginosa* and *Acinetobacter baumannii* are more prevalent (Sakkas *et al*., 2019). In a previous study, Bardaquim *et al*., (2014) explained that 58 % of bacterial contamination is minimized by improving the hygienic conditions of the hospital environment including the patient zones. Nowadays, the reasons of the lower rate of bacterial contamination in several hospitals are the modern technologies, practices of cleaning and disinfection that are currently adopted more than before. The most prevalent bacteria recorded in this study are *Staph. aureus* (40%), *Staph. epidermidis* (12.5%), *E. coli* (10.83%), *Staph. saprophyticus* (8.3%), *K. pneumonia* (7.5%), and *P. aeruginosa* (5.83%). Current results are in accordance with the previous study of Carvalho *et al*., (2009). Similar findings are also observed by Shopsin and Kreiswirth, (2001) who recorded *Staph. aureus* as a multidrug resistant as well as most abundant bacteria in hospitals environment.

In the current work, *Staph. aureus* has shown resistance against multiple numbers of antibiotics such as; Amoxcillin (89.59%), Cefoxime (77.09%), Amikacin (72.9%). In accordance with our findings, Sergent *et al*., (2012) found that *Staph. aureus*, *Acinetobacter baumannii*, *P. aeruginosa*, and Enterobacteriaceae were present in the patient's room, and these bacteria expressed significant resistance against several antibiotics including; Gentamicin, Amikacin, Nitrofurantoin, Cefoxime, and Amoxicillin with and without Clavulanic acid. This may lead to the persistence of these bacteria with multi-drug resistance (MDR) patterns, because of the improper use of such antibiotics without doctor prescription. Aloush *et al*., (2006) previously documented that *P. aeruginosa* is a major cause of hospital acquired infections, and is responsible also for about 10% of all hospitals nosocomial infections. In our study, the prevalence of *P. aeruginosa* is low compared to the previous study of Ijaz *et al*., (2019), because modern techniques used in the different hospitals of Faisalabad for cleaning and disinfection have reduced contamination by such bacterial sp. Moreover, the major sources of contamination by *P. aeruginosa* in our research are door handles and windows, however in the previous study conducted by Aloush *et al*., (2006) it was the wash sinks that considered as the potential sources of this pathogenic bacterium. In the present study, the prevalence of the Gram positive bacteria is more (60.83%) than that of the Gram negative rods (24.13%). Current study highlighted that the bacterial contamination may pose a noticeable negative impact in different hospitals of Faisalabad, thus clinicians and doctors should be keener to find solutions to solve this problem.

Current results demonstrated that most of the bacteria that caused hospital acquired infections (HAIs) are resistant to multiple antibacterial agents. This is consistent with the previous study of Mitchell *et al*., (2015) who also reported that the healthcare workers who wear lab coats and scrubs are considered as the potential sources for bacteria that cause nosocomial infections. Doll *et al*., (2018) reported that the quality of cleaning the hospital surfaces is generally evaluated by estimating the human factors which are considered as the best defensive system of the patients against the invisible threats emerging from the hospital inanimate environment.

Conclusion

The prevalence of the Gram-positive bacteria is more than the Gram negative bacteria on the hospital inanimate surfaces; however, the Gram positive one play significant role in causing nosocomial infections. The Gram-positive bacteria are more resistant to ampicillin and ceftriaxone, whereas the Gram-negative bacteria are more resistant to Norfloxacin. Both types of bacteria are highly sensitive to Ciprofloxacin, Cefoxime and Nitrofurantoin.

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Conflict of interest

The authors declare that there is no conflict of interests.

5. References

Adam, A.S.; Ntulume, I.; Adeyemo, R.; Akinola, S.; Abubakar, I.J.; Aleiro, A.A. and Namatovu, A. (2019). Antibacterial activity of *Carica papaya* against Methicillin resistant *Staphylococcus epidermidis* Isolated from Wards Surfaces of Kampala International University Teaching Hospital, Bushenyi, Uganda. Current Trends in Biotechnology and Pharmacy. 13(4): 391-400.

Aloush, V.; Navon-Venezia, S.; Seigman-Igra, Y.; Cabili, S. and Carmeli, Y. (2006). Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. Antimicrobial agents and Chemotherapy. 50(1): 43-48.

Bardaquim, V.A.; Oliveira-de-Souza, C.W.; de-Melo-Martins, D.; Carlos Alberto Soares, C.A. and de Sousa, C.P. (2014). Microbiological characterization of the surface contamination in surgical room areas in a Hospital in Sao Paulo (Brazil). Infectio. 18(4):130-134.

Cappuccino, J.G. and Sherman, N. (2005). Microbiology: A Laboratory Manual. San Francisco: Pearson/Benjamin Cummings. pp. 507.

Carvalho, K.S.; Melo, M.C.; Melo, G.B. and Gontijo-Filho, P.P. (2009). Hospital surface contamination in wards occupied by patients infected with MRSA or MSSA in a Brazilian university hospital. Revista de Ciências Farmacêuticas Básica e Aplicada. 28(2): 159-163.

Cataño, J.C.; Echeverri, L.M. and Szela, C. (2012). Bacterial contamination of clothes and environmental items in a third-level hospital in Colombia. Interdisciplinary Perspectives of Infectious Disease. 2012: 1-5.

Doll, M.; Stevens, M. and Bearman, G. (2018). Environmental cleaning and disinfection of Patient areas. International Journal of Infectious Disease. 67: 52-57.

Espinal, P.; Marti, S. and Vila, J. (2012). Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. Journal of Hospital Infection. 80(1): 56-60.

Han, J.H.; Sullivan, N.; Leas, B.F.; Pegues, D.A.; Kaczmarek, J.L. and Umscheid, C.A. (2015). Cleaning hospital room surfaces to prevent health care–associated infections: a technical brief. Annals of International Medicine. 163(8): 598-607.

Harris, A.D.; McGregor, J.C. and Furuno, J.P. (2006). What infection control interventions should be undertaken to control multidrug-resistant gram-negative bacteria?. Clinical Infectious Disease. 1; (43 Suppl 2): S57-61.

Humphries, R.M.; Ambler, J.; Mitchell, S.L.; Castanheira, M.; Dingle, T.; Hindler, J.A. and Sei, K. (2018). CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. Journal of Clinical Microbiology. 56(4): e1934-17.

Ijaz, M.; Siddique, A.B.; Rasool, M.H. and Shafique, M. (2019). Frequency of Multi drug resistant *Pseudomonas aeruginosa* in different wound types of hospitalized patients. Pakistan Journal of Pharmaceutical Sciences. 32(2 Supplement): 865-870.

Ilstrup, D.M. (1990). Statistical methods in microbiology. Clinical Microbiology Reviews. 3(3): 219-226.

Jinadatha, C.; Villamaria, F.C.; Coppin, J.D.; Dale, C.R.; Williams, M.D.; Whitworth, R. and Stibich, M. (2017). Interaction of healthcare worker hands and portable medical equipment: A sequence analysis to show potential transmission opportunities. BMC Infectious Disease. 17(1): 800.

Mitchell, A., Spencer, M. and Edmiston, Jr.C. (2015). Role of healthcare apparel and other healthcare textiles in the transmission of pathogens: A review of the literature. Journal of Hospital Infection. 90(4): 285-292.

Rutala, W.A.; Stiegel, M.M.; Sarubbi, F.A. and Weber, D.J. (1997). Susceptibility of antibiotic-susceptible and antibiotic-resistant hospital bacteria to disinfectants. Infection Control and Hospital Epidemiology. 18(6): 417-421.

Saka, K.H.; Akanbi, I.I. A.A; Obasa, T.O.; Raheem, R.A.; Oshodi, A.J. and Kalgo, Z.M. (2016). Pathogenic Aerobic Bacterial Contaminants on Non-Critical Hospital Surfaces within Pediatric Ward of a Nigerian Hospital. Journal of Medical Microbiology and infectious Diagnosis. 5(241): 2161-0703.

Sakkas, H.; Bozidis, P.; Ilia, A.; Mpekoulis, G. and Papadopoulou, C. (2019). Antimicrobial Resistance in Bacterial Pathogens and Detection of Carbapenemases in *Klebsiella pneumoniae* Isolates from Hospital Wastewater. Antibiotics. 8(3): 85.

Sergent, A.P.; Slekovec, C.; Pauchot, J.; Jeunet, L.; Bertrand, X.; Hocquet, D. and Talon, D. (2012). Bacterial contamination of the hospital environment during wound dressing change. Orthopedics and Traumatology: Surgery and Research. 98(4): 441-445

Shopsin, B. and Kreiswirth, B.N. (2001). Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus*. Emerging Infectious Disease. 7(2): 323-326.