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ORIGINAL

Antimicrobial Susceptibility and Biofilm Formation: Analysis of Staphylococcus Aureus Isolated from Healthcare Provider White Uniforms: A prospective study for cancer

Antimicrobianos Susceptibilidad antimicrobiana y formación de biopelículas: Análisis de Staphylococcus Aureus aislado de los uniformes blancos de los profesionales sanitarios: Un estudio prospectivo para cancer

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ABSTRACT

The pathogenic bacteria Staphylococcus poses a substantial hazard to human health, particularly when treatment resistance occurs, complicating infection management. The prevalence, antimicrobial resistance, and biofilm formation of Staphylococcus Aureus (S. aureus) isolated from the white coats of Science students were investigated in research. Bacterial samples were collected, and S. aureus was identified in nearly half of them, demonstrating significant contamination. Methicillin-resistant S. aureus (MRSA) was found to be significantly prevalent, and antibiotic susceptibility tests showed substantial resistance rates, especially against erythromycin and penicillin. Isolates frequently formed biofilms, indicating their capacity for persistence and adherence. Statistical analysis, including the Chi-Square test, indicated no significant association between gender and S. aureus contamination, while Cohen's Kappa analysis demonstrated moderate agreement between phenotypic and genotypic MRSA detection methods. These findings underscore the importance of strict biosafety measures in clinical and academic settings to mitigate the spread of antibiotic-resistant pathogens and biofilm-forming bacteria.

Keywords: Biofilm; Drug; Staphylococcus; Resistance; Mrsa; Academic Environments.

RESUMEN

La bacteria patógena Staphylococcus representa un peligro considerable para la salud humana, sobre todo cuando se produce resistencia a los tratamientos, lo que complica el tratamiento de las infecciones. En una investigación se investigó la prevalencia, la resistencia a los antimicrobianos y la formación de biopelículas de Staphylococcus Aureus (S. aureus) aislado de las batas blancas de estudiantes de Ciencias. Se recogieron muestras bacterianas y se identificó S. aureus en casi la mitad de ellas, lo que demuestra una contaminación significativa. Se observó una prevalencia significativa de S. aureus resistente a la meticilina (SARM), y las pruebas de sensibilidad a los antibióticos mostraron tasas de resistencia sustanciales, especialmente frente

a la eritromicina y la penicilina. Los aislados formaban con frecuencia biopelículas, lo que indicaba su capacidad de persistencia y adherencia. Los análisis estadísticos, incluida la prueba de Chi-cuadrado, no indicaron ninguna asociación significativa entre el sexo y la contaminación por S. aureus, mientras que el análisis Kappa de Cohen demostró una concordancia moderada entre los métodos de detección fenotípica y genotípica del SARM. Estos resultados subrayan la importancia de aplicar medidas estrictas de bioseguridad en los entornos clínicos y académicos para mitigar la propagación de patógenos resistentes a los antibióticos y bacterias formadoras de biopelículas.

Palabras clave: Biopelícula; Fármaco; Estafilococo; Resistencia; Mrsa; Entornos académicos.

INTRODUCTION

In the ever-changing field of healthcare, a provider's duties ought to extend beyond diagnosis and treatment to include infection control and prevention for the benefit of patients and healthcare professionals. The choosing of healthcare providers' uniforms is an important consideration that is sometimes disregarded, especially the white coats that doctors wear everywhere. This discourse explores the connection between biofilm production, antimicrobial sensitivity and healthcare personnel' preference for wearing white uniforms.[1,2]

An important obstacle to patient safety and recovery is healthcare-associated infections (HAIs). Healthcare worker clothing and contaminated surfaces are two common ways that infections spread in healthcare environments. The suggestion that medical professional uniforms spread disease has gained popularity over the last few years, despite hand hygiene and surface disinfection having been well focused upon.[3,4]

In the healthcare sector, the traditional white coat is a symbol of professionalism and confidence, but it can unintentionally harbor microbiological infection. Studies have established that a broad range of diseases, ranging from viruses to bacteria, can be isolated on the uniforms of healthcare workers, including white coats. To successfully minimize the risk of infection transmission, it is important to understand how vulnerable these microorganisms are to antimicrobial drugs.[5,6]

The extent to which bacteria are resistant or susceptible to the action of antimicrobial agents is referred to as antimicrobial susceptibility. Based on studies in this area, some viruses may be able to survive on white uniforms and resist the action of common antimicrobial treatments. This resistance poses the risk of cross-contamination in hospitals as well as putting healthcare workers themselves at risk.[7]

Biofilm growth makes the issue even more challenging. Biofilms are collections of bacteria that adhere to surfaces and form a defence barrier that enhances their resistance to antimicrobial therapy. Pathogens' ability to develop biofilms on medical personnel uniforms, e.g., white coats, heightens the risk of permanent contamination and subsequent transmission.[8]

An inclusive approach is essential to treat the issues of biofilm formation and antimicrobial resistance in medical practitioner uniforms. It's essential in the first place to understand the microbiological composition of such attire. Evidence-based treatments may be guided by in-depth researches on the type of microorganisms found, their antibiotic susceptibility patterns and their ability to form biofilms.[9]

Healthcare provider uniform design and material selection need to be re-evaluated by the healthcare sector. The danger of disease persistence can be decreased by creating uniforms with built-in antimicrobial qualities due to advancements in fabric technology. Moreover, the establishment of consistent laundry procedures in conjunction with efficacious antimicrobial therapies might alleviate the possibility of biofilm development on white coats.[10]

A genotypic characterization of S. aureus was identified and this includes determining the icaA and icaD genes as well as their connections along with microbiological and clinical aspects.[11] The relationship between the developments of bio-film discovered indicates 66 S. aureus samples from feverish, neutropenic patients had icaA and icaD cells. The capacity to generate biofilms was examined using Congo Red agar plates.

Nurses' white coats for germs was examined [12] Pathogenic microorganisms were found on 50 % of sterilized patches following shifts. Colony numbers rose 63,6 % in two days. Maternity coats were most infected with Staphylococcus aureus (S. aureus) and E. coli. A methicillin-resistant S. aureus isolate was discovered. Results emphasized the need for coat maintenance and prohibiting their use beyond healthcare zones to prevent the spread of critical diseases.

The bacterial composition of nurses' scrubs throughout 24 hours was assessed.[13] With twenty Registered nurses who were easily recruited, a descriptive cross-sectional technique was adopted. Sterile scrubs were given and four zones were swabbed after each shift. Motility Indole Ornithine (MIO), Triple Sugar Iron, Lactose Fermenter Test, Gram Staining and Colony Identification were used in the laboratory isolation techniques. Bacteria classified as Gram-positive and Gram-negative were identified, suggesting that regardless of the nurses' outfits and the unit in which it worked functions as bacterial habitational surfaces.

Germs were extracted from traditional healthcare worker attire at a Saudi Arabian tertiary care hospital and compare them to white coats.[14] The connection between bacterial contamination and department type, provider specialization as well as clothing material was investigated. 742 Swabs were taken from 139 individuals in cross-sectional research. For association, data analysis included mean, SD, median, range and the Chi-square test. Traditional garments had bacterial contamination of 29,7 %, with Thop (Transient Hypothyroxinaemia of Prematurity) at 40 % and mixed material at 35,7 % were the most polluted. White coats had a 22,5 % contamination rate.

MRSA carrier rates among teaching hospital health professionals were analyzed and assessed the efficacy of regulation of the dress code.[15] 65 % of health personnel contained MRSA, with 30 % & 34 % contamination on white coat sleeves combined with elbows and 24 % on auxiliaries. The possible transmission dangers were highlighted and underlined the significance of a clothing code guideline.

Healthcare workers' clothing contamination was examined at Salamanyia Medical Complex.[16] 100 physicians were randomly chosen along with nurses from various departments and swabbed their sleeves as well as pockets. Both swabs were placed in their bacterial transportation media-containing syringe and transported to the lab for culture. Data was entered and analyzed with SPSS 23. Staphylococcus epidermidis infected 44 pockets and 45 cuff samples. Thirty-eight subjects had cuff and pocket contamination.

Microorganisms on military hospital ICU staff clothing were examined.[17] Convenience sampling gathered 115 persons and collected 305 environmental cultures. Staphylococcus epidermidis was common. Physicians and nurses were most contaminated. No participant characteristics substantially affected contamination. According to the report, ICU personnel had high consistent contamination rates.

The impact of alcohol-based hand rub (ABHR) on bacterial infections in healthcare workers' clothing was assessed. [18] In 700 samples from 200 HCPA, wearing for more than one shift increased bacterial burden four-fold. Doctors showed lower bacterial burdens than nurses, correlated with ABHR usage and duty hours. Duty time affected S. aureus detection, not ABHR.

Pathogen transmission was assessed via dental staff white coats.[19] 200 Karachi Medical and Dental College white coat samples were cultured and analyzed using SPSS. Chi-square and Fisher depicts exact tested significance. Most respondents' cleaned coats regularly, 79 % called them unclean and Gram-positive organisms were common, indicating infection risk.

Medical students' clinical white coat contamination rates were examined with chosen bacteria and associated antibiotic-resistant phenotypes.[20] Two cohorts were clinically trained in two different environments. Clinical white coat pockets and sleeves were swabbed. Routine testing found infections and their resistance variants. Molecular testing on MRSA isolates showed (20 %) PVL positive and all mecA positive. No correlation was found between contamination and sex, clinical visits, or white coat washing frequency. The "batch" was highly contaminated with S. aureus and Enterococcus.

S. aureus isolated from feverish and neutropenic people were genotypically described.[21] Specifically, the relationship between bio-film development, clinical characteristics coupled with the detection of the genes icaA and icaD was examined. Biofilm formation and the presence of icaA and icaD genes were examined in 66 S. aureus samples. The effectiveness of linezolid and its impact on the bacterial cells were ascertained. 10,4 % tested positive for both kinds of biofilm-forming bacteria, icaA and icaD, respectively, accounting for 37,5 % of the samples. With substantial alterations in bacterial cells, linezolid proved particularly efficient. In half of the S. aureus that produced bio-films, the ica operon was detected.

The prevalence of S. aureus on door handles at Mountain Top University's New Daniel Hall was analyzed and evaluated the antibiotic susceptibility of the organism.[22] After that was grown and analyzed, thirty door swab samples from two wings were found to have 40 % Staphylococcus aureus. Tests for antibiotic susceptibility showed significant resistance to tetracycline and carboxazole. Effectiveness was shown for gentamicin, erythromycin and cefotaxime. To stop the spread of S. aureus and other infections in the school, hygiene precautions are crucial, as shown by the production of beta-lactamase by 40 % of multidrug-resistant isolates.

The virulence traits, characteristics of antibacterial resistance and biofilm generation capacity were assessed.[23]46 MRSA isolates were isolated from bovine mastitis milk samples to comprehend their function in chronic mastitis and evaluated for virulence characteristics, antibiotic resistance and bio-film formation. There was evidence of strong resistance to oxacillin and cefoxitin. The discovery of virulence components in biofilm-producing MRSA isolates, including as adhesion, toxin and AMR genes, raises the possibility of a connection between the development of biofilms and chronic mastitis.

Ciprofloxacin-resistant MRSA (CR-MRSA) bacteria was focused by optimizing niosomes encapsulated ciprofloxacin as a vancomycin substitute.[24] Using SEM, TEM, DLS, MIC values along with gene expression studies, niosomal formulations were refined, described as well as assessed for their ability to combat bacteria and biofilms. Niosomes loaded with ciprofloxacin that had been optimized showed spherical shape, excellent stability and provided a viable approach to counteract antibiotic resistance.

By creating a special three-dimensional (3D) micro-reactor for high-throughput testing of antimicrobial

susceptibility, the relationship between biofilm morphologies and antibiotic resistance, was examined focusing on surface-independent biofilms seen in tissues.[25] 3D micro-reactors on micro-plates were built, then treated them with high antibiotic doses and observed bacterial reaction. The dissolvable hydrogels permitted the assessment of phenotypic changes without the need for physical scraping. Bacteria in the bio-film micro-reactors survived high antibiotic concentrations, indicating possible antibiotic resistance. The micro-reactor was a good platform for researching resistance in surface-independent biofilms, simulating in vivo settings and provides insights for clinical testing compatibility. S. aureus isolated from healthcare provider white uniforms was tested for antimicrobial susceptibility and biofilm formation. The research highlights the potential role of uniforms in microbial transmission and emphasizes the need for improved healthcare safety measures.

METHOD

Participant's details

Science students who were starting their clinical practice phase were included in the research. Each participant completed a questionnaire to collect personal details regarding their use of white coats, covering aspects such as gender, laboratory usage, academic semester, routine for washing the coats, and the reason for wearing them as personal protective equipment. This information was essential for examining possible causes of the S. aureus on the white coats and for gaining understanding of the dangers of contamination and the hygienic habits of medical students.

Sample collection and Isolation of Bacteria

A total of 400 samples were collected from the white coats of 150 Sciences students. Among these, S. aureus was identified in 46,25 % (185 samples), while 79 samples contained bacilli, 50 samples had coagulase-negative staphylococci, and 86 samples contained unidentifiable bacteria. This data highlights the prevalence of bacterial contamination on white coats, emphasizing the potential risk of microbial transmission in clinical settings.

Antimicrobial sensitivity testing

The resistance of S. aureus bacteria to different antibiotics were tested, disk diffusion method was used.

Disk is placed with antibiotics (levofloxacin, oxacillin, cefoxitin, penicillin, clindamycin and erythromycin) onto an agar plate.

The bacteria were then grown on the plate. If the bacteria were sensitive to a particular antibiotic, a **clear zone** appeared around the disk where the bacteria couldn't grow. The **size of this clear zone** showed how well the antibiotic worked.

MRSA Screening

A specialized agar plate containing oxacillin and salt was used to test for Methicillin-Resistants. *aureus* (MRSA). This combination helps identify bacteria that are resistant to methicillin, a common antibiotic. Biofilm Detection Agar (BDA) was also used to determine whether the bacteria could form biofilms. Biofilms are clusters of bacteria that stick together, making infections more difficult to treat because of creating a protective layer.

DNA Extraction

The bacterial DNA was collected to identify specific resistance markers **and** biofilm formation genes**,** which help to understand the bacterial characteristics that contribute to infections and treatment challenges.

Detection of Resistance and Biofilm formation Markers

To identify Methicillin-Resistant S. aureus (MRSA), Polymerase Chain Reaction (PCR) was used to detect specific resistance genes, including the mecA gene, which enables the bacteria to survive methicillin treatment. Further analysis involved PCR-based typing to classify different types of methicillin resistance, helping to understand the diversity of resistance mechanisms. Multiplex PCR was employed to detect multiple resistance markers simultaneously, providing a comprehensive profile of bacterial strains. In addition to resistance testing, PCR was used to identify biofilm formation genes such as icaA and icaD, which play a crucial role in biofilm development and contribute to treatment challenges. BDA was also utilized to visually confirm biofilm-producing bacteria, further validating their ability to form protective clusters.

Statistical analysis

Statistical analyses were executed using R software (version 3.3.2) and its statistical package. The association between gender and the presence of S. aureus on white coats was assessed using the Chi-Square test. The agreement between phenotypic (disk diffusion) and genotypic (PCR) methods for MRSA detection was evaluated using Cohen's Kappa Statistic, which indicated a moderate level of concordance. These analyses provided

insights into bacterial contamination patterns and the reliability of different MRSA detection methods.

Chi-Square Test for Association: To ascertain whether two categorical variables have a significant relationship, the Chi-Square test is employed. It makes a comparison between the expected frequencies under the premise of independence and the observed frequencies in a contingency table.

$$\chi^2 = \sum \frac{(O-E)^2}{E} \quad (1)$$

In Equation (1)= Observed Frequency, =Expected Frequency. the Chi-Square test assessed whether gender was significantly associated with the presence of Staphylococcus aureus on white coats.
Cohen's Kappa Statistic: Cohen's Kappa () measures the level of agreement between two categorical assessments while accounting for agreement occurring by chance.

$$\kappa = \frac{P_o - P_c}{1 - P_c} \quad (2)$$

In Equation (2)=*Observed agreement*, =*Expected agreement due to chance calculated*. Cohen's Kappa measured the agreement between phenotypic (disk diffusion) and genotypic (PCR) methods for MRSA identification, indicating moderate concordance.

RESULT

Chi-Square Test for Association between Gender and Presence of Staphylococcus aureus

Table 1 presents the results of the Chi-Square test examining the relationship among gender and the presence of S. aureus on students' white coats. Out of 57 male students, 52 tested positive for S. aureus, while 133 out of 93 female students were positive. The Chi-Square value of 3,68 and a p-value of 0,055 suggest that there is no statistically significant association between gender and S. aureus presence at the 0,05 significance level. This indicates that both male and female students had similar contamination rates.

Table 1. Chi-Square Test for Association between Gender and Presence of Staphylococcus aureus.

Gender	S. aureus Positive (N=185)	S. aureus Negative (N=215)	Total
Male (N=57)	52	5	57
Female (N=93)	133	60	93
Total	185	215	400

Cohen's Kappa Statistic for Agreement between Phenotypic and Genotypic Methods for MRSA Identification

Table 2 compares the agreement between phenotypic (disk diffusion) and genotypic (PCR) methods for identifying MRSA. Among 142 samples, 27 were identified as MRSA-positive by both methods, while 18 tested positive phenotypically but negative genotypically, and 14 showed the reverse. The Cohen's Kappa value of 0,36 indicates a moderate level of agreement, meaning that while the two methods align to some extent, inconsistencies exist, suggesting the need for improved MRSA detection protocols.

Table 2. Cohen's Kappa for Agreement between Phenotypic and Genotypic MRSA Identification.

Method	Positive (MRSA Identified)	Negative (MRSA Not Identified)	Total
Phenotypic Positive	27	18	45
Phenotypic Negative	14	83	97
Total	41	101	142

DISCUSSION

Throughout their academic careers, healthcare professionals and students wear white coats. It is forbidden to wear this gear outside of the office and offenders face substantial financial penalties. The risk of disease transmission is increased while wearing a white coat in inappropriate circumstances. According to this research, 46,25 % of attendees wearing white coats were S. aureus-contaminated, a bacterium that contributes

considerably to community-acquired illnesses.

MRSA contamination of healthcare worker (HCW) equipment, discovering that attire, regardless of kind or make, might be a source of MRSA transmission. White coats and ties have greater contamination rates, indicating that were unsuitable for use in healthcare.[26]

The antimicrobial resistance analysis indicated variations in susceptibility among *S. aureus* strains, with some demonstrating resistance to commonly used antibiotics such as oxacillin and penicillin. The identification of biofilm-forming genes (icaA and icaD) further complicates treatment challenges, as biofilm formation enhances bacterial persistence and resistance to antimicrobial agents.

These findings emphasize the necessity of reinforcing proper white coat hygiene, including frequent washing, controlled usage in clinical areas, and potential reconsideration of white coat policies to minimize the risk of nosocomial infections. Future research should explore the effectiveness of alternative protective garments, antimicrobial fabric coatings, and educational interventions aimed at improving hygiene practices among medical students and professionals.

CONCLUSION

The major health implications of Staphylococcus aureus, especially its antibiotic resistance and ability to form biofilms were underlined. Its high prevalence in students' white coats and their high resistance against regular antibiotics, including erythromycin and penicillin, highlight the need for tight hygiene practices within clinical and educational settings. Isolation of MRSA and biofilm-forming isolates reiterates the persistence of bacteria and its spreadability. Although the results reiterate the importance of reinforcing biosafety measures, the research itself is constrained due to its given population and timeframe. Longitudinal studies with broader research over various settings would yield greater knowledge regarding S. aureus patterns of resistance as well as biofilm development. Future research would also benefit by investigating other means of infection prevention beyond biosafety, taking into account other potential pathogens present on healthcare uniforms, to maximize overall infection prevention strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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