Antibiotic Sensitivity Pattern of *Staphylococcus aureus* Isolated from Pus Samples of Different Age and Sex Groups in Gazipur District, Bangladesh

Md. Eaktear Uddin[1], Shakila Sultana[1], Maruf Abony[1], Suvamoy Datta[1]*

Abstract

Staphylococcus aureus has long been reported as one of the most important bacteria that cause different kinds of diseases in humans, particularly skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. To treat these infections, certain antibiotics have frequently been used across the world. However, nowadays, there is a growing concern on the treatment of staphylococcal infections due to gaining resistance to some antibiotics by this bacterium. In the present work, S. aureus was isolated from 40 pus samples and a total of 40 isolates were selected to study their multi-drug resistance pattern using 10 available antibiotics. . Among the antibiotics used, gentamycin was found to be the most effective to control S. aureus as the isolates showed minimum resistance to this antibiotic (0%). On the other hand, the isolates showed the highest resistance to amoxicillin (100%). Among 40 isolates, one isolate (2.5%) was resistant to the maximum number of antibiotics used in the experiments (seven), while five isolates (12.5%) showed resistance to maximum 6 antibiotics, and five isolates (12.5%) were resistant to at least 5 antibiotics. Overall, a total of 27.5% isolates were multi-drug resistant (resistant to 5 or more of the antibiotics tested). In conclusion, the findings of this study would be helpful to choose appropriate antibiotic for treating staphylococcal infections considering the multi-drug resistance of the bacterium.

Keywords: S. aureus, Pus samples, Gazipur city, Bangladesh.

Significance | *S. aureus* infections pose a global health threat with rising antibiotic resistance. Gentamycin emerges as a crucial treatment amidst multidrug resistance challenges.

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Introduction

The global rise in antibiotic resistance poses a significant threat to public health, driven by factors such as overuse and misuse of antibiotics, limited new drug development, and environmental impacts. Antibiotics, once hailed as miracle drugs, are increasingly losing effectiveness against bacterial infections due to the development of resistant strains. This crisis has prompted declarations from major health organizations, including the CDC and WHO, underscoring its severity and urgent need for action (Bartlett, Gilbert, & Spellberg, 2013). Antibiotic resistance emerges through various mechanisms, including genetic inheritance and horizontal gene transfer (HGT) among bacteria, as well as spontaneous mutations (Bauer, 1966). The misuse of antibiotics in human medicine, such as overprescription and incorrect usage, exacerbates this problem (Blot et al., 2002; Carter et al., 2000). Furthermore, antibiotics used as growth promoters in livestock contribute significantly to antibiotic resistance, with a large portion of antibiotics administered to animals eventually entering the human food chain and environment (Golkar, Bagasra, & Pace, 2014). The consequences of antibiotic resistance are profound,

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affecting treatment outcomes for infections caused by both gram-positive and gram-negative bacteria. Methicillin-resistant *Staphylococcus aureus* (MRSA) is emblematic of this challenge, causing severe infections in hospital and community settings alike (Holden, 1984). Initially treatable with β-lactam antibiotics, MRSA strains evolved to produce the mecA gene, conferring resistance to these drugs through altered penicillin-binding proteins (PBP2a or PBP2') (Zhanel & Mendem, 2016). This resistance necessitates the use of alternative antibiotics like glycopeptides, yet even these are now challenged by emerging strains of vancomycin-resistant S. aureus (VRSA) (Rossolini et al., 2014). In healthcare settings, gram-negative bacteria such as Klebsiella pneumoniae and Pseudomonas aeruginosa also pose significant challenges due to their multidrug resistance (MDR) profiles, often acquired through mechanisms like extended-spectrum beta-lactamase (ESBL) production and efflux pumps (Hiramatsu et al., 1997). The treatment options for these infections are increasingly limited, relying on last-resort antibiotics that are often less effective, more toxic, and costly. In response to the growing crisis, research efforts are focusing on developing new antibiotics and alternative treatment strategies. However, the pharmaceutical industry faces obstacles such as reduced economic incentives and stringent regulatory requirements, which hinder innovation in this critical area (Bartlett, Gilbert, & Spellberg, 2013). The urgency of the situation demands international collaboration and sustained investment in novel antimicrobial therapies to combat the evolving landscape of antibiotic resistance. Bangladesh, like many lower-middle-income countries, is also grappling with the challenges posed by antibiotic-resistant infections. The prevalence of antibiotic resistance patterns among bacterial isolates, including S. aureus, is not only a local concern but also a global health issue due to the potential for international spread of resistant strains (Ventola, 2015). Studies assessing antibiotic resistance profiles in such settings are crucial for informing local treatment guidelines and global surveillance efforts. This study aims to analyze the prevalence of S. aureus infections and antibiotic resistance patterns in Gazipur, Bangladesh. Pus samples collected from various sources will be examined to determine the susceptibility of S. aureus isolates to different classes of antibiotics commonly used in clinical practice. Understanding the local epidemiology of antibiotic resistance is essential for optimizing treatment strategies and preserving the effectiveness of existing antibiotics. The methodology will involve conducting antibiotic susceptibility tests using standard procedures recommended by international guidelines, ensuring robust and comparable data. Results will provide insights into which antibiotics remain effective against local strains of S. aureus and identify any emerging resistance patterns that require attention. This information is critical for healthcare providers in Bangladesh to make informed decisions regarding empirical treatment and infection control measures.

The antibiotic resistance crisis represents a formidable challenge to global health, necessitating coordinated efforts across sectors and countries to mitigate its impact. Research initiatives and surveillance programs are essential for monitoring resistance trends and developing strategies to preserve antibiotics' effectiveness for future generations. By addressing the drivers of resistance and enhancing stewardship of these valuable drugs, we can hope to sustain their efficacy in combating infectious diseases worldwide.

Materials and Methods

Sample collection

A total of 40 clinical specimen (pus) were collected from diagnostic and health care centers from different places at Gazipur in Bangladesh, via. Transport media. All sample containers were marked immediately after sample collection and were transported to the laboratory within 1 hour. The bacterial count was performed by standard method. The microbiological condition of safety and hygiene were then assayed using the methods recommended by ICNSF.

Mannitol salt agar (MSA) medium was used for this purpose. Homogenate sample (0.1 ml) from each dilution was taken on to each sterile petridish. Then the sample was homogeneously distributed on the plate using a glass spreader in a backward and forward movement while rotating the plate. Then the plates were incubated at 37°C for 24 hours. Biochemical studies of the isolated bacterial strains Biochemical tests include Catalase test, IMVic test (Indole production, Methyl Red, Voges Proskauer, Citrate Utilization test) were carried out for the characterization and identification of the bacterial isolates as per standerd protocols (Viswanathan et al, 2014). The presence of the enzyme catalase in the rhizobial isolates was examined by suspending one loopful of organism in a drop of 3% H2O2 on a glass slide. Production of bubbles indicates a positive result. A discrete colony was picked by a sterile loop and immersed into SIM agar in the tube. After addition of Kovac's reagent, the formation of cherry red layer indicated the positive results. The Methyl Red test was done by picking a pure colony by a sterile loop and immersing it in the MR–VP broth. Then 5 drops of Methyl red indicator was added. Formation of red color indicated the positive result. Voges Proskauer Test was done by picking a pure colony by a sterile loop and immersing it in the MR–VP broth. Then 10-15 drops Barritt's reagent was added and vigorously shaken. Formation of rosy pink color after half an hour indicated the positive result. A discrete colony of the isolate was picked and streaked onto the slant surface of Simmon's Citrtate Agar medium. After overnight incubation at 37°C, the medium turned Prussian blue from green for a positive result.

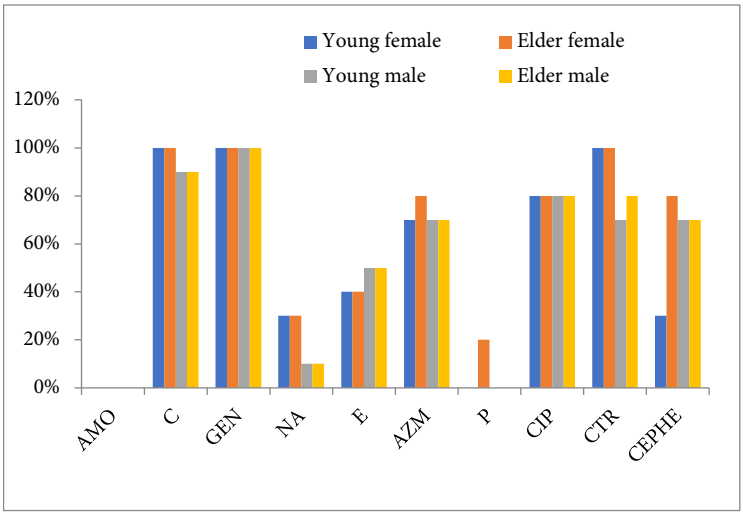

Figure 1. Comparative analysis of sensitivity (%) of S. aureus to antibiotics among different age and sex groups.

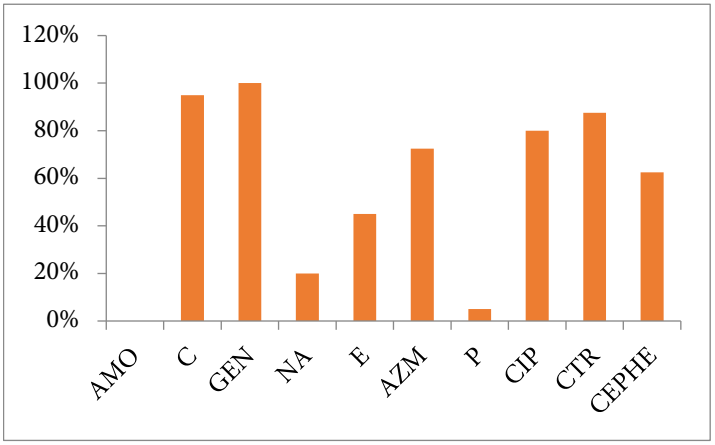

Figure 2. Overall sensitivity pattern (%) of S. aureus to each antibiotic in different age and sex groups.

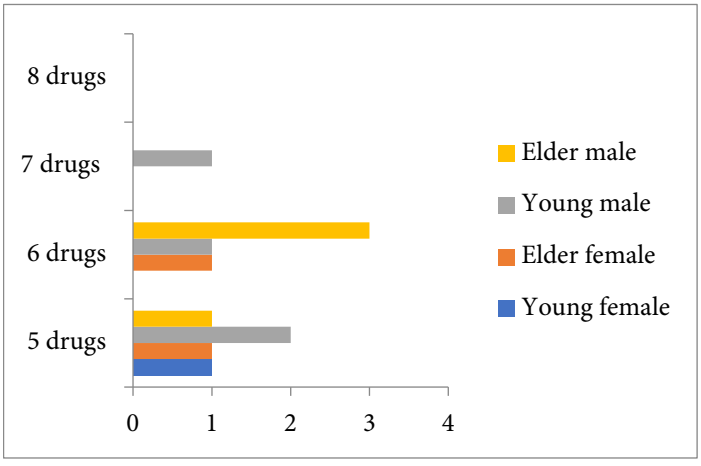

Figure 3. Multidrug resistance (%) patterns of S. aureus in different age and sex groups.

For determining susceptibility of *Staphylococcus aureus* against antimicrobial agent, in vitro agar disc-diffusion method was used which is known as the Kirby Bauer method (Zhanel et al, 2010). A total of six commercially available antibiotic discs were studied that include amoxycillin (AMO), chloramphenicol (C), gentamycin (GEN), nalidixic acid (NA), erythromycin (E), azithromycin (AZM), penicilin (P), ciprofloxacin (CIP), ceftriaxone (CTR), cepheradine (CEPHE). A suspension of the test organism was prepared containing 103 to 105 cfu/ml. Turbidity of the culture broth was adjusted with normal saline to match the equivalent turbidity standard of McFarland (0.5 Standard). A sterile cotton swab was used to spread the bacterial suspension evenly over the entire surface of a Mueller-Hinton agar (pH 7.4) plate for obtaining uniform inoculum. Antibiotic discs were placed aseptically using sterile forceps on the surface of the inoculated plates and incubated at 37°C for 18 to 24 hours.

Results and Discussion

Out of the 40 pus samples collected from various locations across Gazipur, all tested positive for the presence of S. aureus. This bacterium, known for its ability to cause a range of infections, was isolated and subjected to biochemical testing to determine its antibiotic sensitivity profile.

The results revealed significant patterns in antibiotic susceptibility among the S. aureus isolates as shown in figure 1. Notably, all isolates showed 100% sensitivity to Gentamycin, indicating it as the most effective antibiotic against this strain. Ciprofloxacin exhibited an 80% sensitivity rate, making it another viable treatment option. Conversely, none of the isolates were sensitive to Amoxycillin, highlighting its ineffectiveness against the sampled strains. Further analysis of antibiotic sensitivity across different demographic groups revealed interesting trends. Chloramphenicol, Nalidixic acid, and Ceftriaxone showed higher sensitivity rates among female groups compared to male universal among the isolates, underscoring its lack of utility in treating S. aureus infections.

Multidrug resistance analysis further highlighted concerning trends within the sampled population. A small percentage (2.5%) of isolates exhibited resistance to seven different antibiotics, indicating a high degree of resistance as shown in figure 2. Additionally, 12.5% of isolates were resistant to six

groups. In contrast, isolates sensitive to Erythromycin were more prevalent among male groups.

The overall resistance patterns of S. aureus strains to the tested antibiotics ranged widely. Gentamycin showed the lowest resistance rate at 0%, reinforcing its efficacy as a primary treatment option. Conversely, resistance to Amoxycillin was

antibiotics, and another 12.5% were resistant to at least five antibiotics. This underscores the emergence of multidrug-resistant strains of S. aureus in the Gazipur region, posing challenges for treatment strategies.

Demographic analysis of multidrug-resistant isolates revealed that resistance was more prevalent among elder and young male groups compared to their female counterparts as shown in figure 3. This observation suggests potential demographic and possibly behavioral factors contributing to the spread of resistant strains. The study provides valuable insights into the prevalence and antibiotic susceptibility of S. aureus isolates in Gazipur. The findings emphasize the critical importance of antibiotic stewardship and surveillance programs to monitor and combat the rise of multidrug-resistant bacteria. Effective treatment strategies should prioritize antibiotics such as Gentamycin and Chloramphenicol, which demonstrated higher sensitivity rates. Continued research and public health efforts are essential to mitigate the impact of antibiotic resistance and ensure effective treatment of S. aureus infections in the region and beyond.

Conclusion

Pus infection poses significant challenges for healthcare providers due to its impact on patient well-being, financial resources, and the demand for cost-effective management. In this study, *Staphylococcus aureus* was the predominant microorganism isolated from pus samples (40 out of 40), highlighting its prevalence in incision-related infections and wound types. Antibiotic susceptibility testing indicated high sensitivity of S. aureus to Gentamycin (100%) and Chloramphenicol (95%), suggesting effective treatment options. Sensitivity patterns varied across age and sex demographics, with concerning trends in multidrug resistance. Addressing these challenges requires coordinated efforts, including policy reforms, enhanced research initiatives, and proactive crisis management strategies. While our study did not explore the impact of hygiene on wound infections, we recommend patient education on personal hygiene to improve wound healing outcomes and patient care management. Efforts to implement these recommendations are crucial for mitigating the impact of pus infections in healthcare settings.

Author contributions

S.D., conceptualized and developed the methodology, M.E.U., prepared the original draft and collected data, M.A., reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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