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Plasmid Curing of Bacteria Isolated from Upper Respiratory Tract Infections among Patients Attending Specialist Hospital Sokoto

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Abstract

Presences of resistant plasmids (R-plasmids) in microorganisms make the cells avert the effect of antibiotics and complicates chemotherapy of infections. This study aimed to cure the plasmid of antibiotic-resistant bacteria associated with upper respiratory tract infections among patients attending Specialist Hospital Sokoto by reassessing their susceptibility to antibiotics that were previously resistant. One hundred (100) throat swab samples were collected and analysed. The isolates were isolated and identified using standard methods (Gram staining, biochemical and serological tests). The susceptibility of the isolates to various beta-lactam antibiotics was evaluated, and resistant bacteria were subjected to plasmid curing experiments followed by further susceptibility testing to reassess their susceptibility to the erstwhile resistance. Bacteria isolated were *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, with the frequency of occurrence of 20 (57.2%), 9 (25.7%), and 6 (17.1%), respectively. The result of antibiotic susceptibility tests before plasmid curing showed that *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* resisted Cloxacilline, Cefuroxime, Cloxacillin and Augmentin. Susceptibility results after curing showed that almost all bacteria have reverted to sensitivity to all antibiotics except Cloxacillin and Augmentin. This research implies that the resistance possessed by the bacterial isolates is plasmid-mediated and may easily be transferred to other non-resistant bacteria, which may lead to an alarming rate of antimicrobial resistance in the study area.

Keywords: Antibiotic resistance, Beta-lactam antibiotics, Plasmid curing, upper respiratory tract infection.

INTRODUCTION

Plasmids are independent, circular, self-replicating extra-chromosomal DNA elements with characteristic copy numbers within the host. Various properties encoded by plasmid include resistance to antibiotics and heavy metals, degradation of hydrocarbons, synthesis of bacteriocins and antibiotics, etc. Plasmid-mediated antibiotic resistance can be transferred easily from one bacterium to another by transformation, conjugation, or mobilization (Patwardhan *et al.*, 2018). Plasmid-encoded resistance to multiple antibiotics has been increasingly recognized as a major challenge in treating infections. In addition to antibiotic resistance, some bacterial

plasmids confer pathogenicity to the host cell (Patwardhan *et al.*, 2018).

Upper Respiratory Tract Infections has been regarded as a nonspecific term that is used to describe acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi (Peroš-Golubičić and Tekavec-Trkanjec, 2015). Bacterial pathogens sometimes serve as the primary causative agents of acute upper respiratory infections, but more frequently, they cause chronic infections. Upper respiratory tract infections (URTIs) are defined as acute febrile illnesses presenting with cough, coryza, sore throat, or hoarseness, which are very common in the community and are one of the major reasons for visiting primary care

physicians, particularly during the winter season (Peroš-Golubičić and Tekavec-Trkanjec 2015). Airborne transmission via droplets and aerosols enables some bacteria to spread efficiently among humans, causing difficult-to-control outbreaks. (Kutter *et al.*, 2018).

Respiratory bacterial pathogens associated with reduced susceptibility to multiple classes of antibiotics include *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis*. Bacteria may acquire antibiotic resistance through the following mechanisms; active efflux of the antibiotics, decreased cell membrane permeability, modification of drug target, or inactivation of the antibiotics (Miriti *et al.*, 2023).

In Nigeria, most upper respiratory tract infections are treated empirically, possibly because of the higher cost of laboratory services. In addition, these diseases are difficult to prevent due to the ease of spreading the infection from person to person or even to the larger community. The emergence of antibiotic resistance in the management of upper respiratory tract infections is a serious public health issue, particularly in the developing world. Apart from high levels of poverty, ignorance, and poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation (Taura, 2021). This research provides information on bacteria associated with upper respiratory tract infections, their susceptibility to routine antibiotics, and assesses bacterial plasmid (R-factor) that is responsible for antibiotic resistance and the possibility of reversing the resistance patterns through sub-inhibitory concentration of acridine orange and equally determines if the resistance is reverted. This study aims to cure antibiotic-resistant bacteria associated with upper respiratory tract infection cases among patients attending Specialist Hospital Sokoto.

MATERIALS AND METHODS

Study Area

Sokoto state is located in the extreme Northwestern part of Nigeria between the longitudes 8°E and 54°E and latitudes of N and 58°N. It forms boundaries with the Niger Republic to the north, Kebbi state to the west and southwest, and Zamfara to the east. The study was carried out in Specialist Hospital Sokoto, Sokoto State, Nigeria. It is located in Sokoto South and was established by the colonial

masters since 1937. It is one of the major referral centers for a number of privately owned hospitals and local government's primary health care within the state. Most of the patients seen at the hospital come from the city metropolis and surrounding districts. Hence, it was suitable to use the center as a study area (Gambo *et al.*, 2017).

Sample Collection

The throat swabs of 100 patients attending Specialist Hospital Sokoto with upper respiratory tract infections were collected using a sterile swab stick by a Medical Doctor. The handle of a spoon was used to depress the tongue to examine the throat for the presence of exudates or pus. The mucous membrane of the throat was rubbed with a sterile swab stick. The swab samples were transported to the laboratory for analysis (Mawaddah *et al.*, 2020).

Isolation and identification of Bacteria

The samples collected were aseptically inoculated onto the surface of prepared nutrient, blood, and chocolate agar plates. This was done by streaking the swab stick on the surface of the agar. The nutrient, blood, and chocolate agar plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for growth. The colonies on the plates were subcultured to obtain a pure culture. All the bacteria isolated were identified based on their morphological characteristics, microscopical appearance, and biochemical and serological tests, according to Gambo *et al.* (2017).

Antibiotic Susceptibility Testing

The sensitivity of the isolates to the beta-lactam antibiotics (Cefuroxime 30µg, Ceftriaxone 30 µg, Ceftazidime 30 µg, Cefoxitin 30 µg, Augmentin 30 µg and Cloxacillin 5 µg) was determined using commercially prepared antibiotics disc. Well-isolated colonies were picked and emulsified in nutrient broth using a sterile wire loop. The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equivalent suspension. Then, 0.1 ml of the standardized inoculum of the bacteria was inoculated and spread evenly on the prepared and dried Mueller Hinton agar plate. Sterile forcep was used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37°C for 24 hours. After the incubation period, observation of the zone of inhibition was done. The

diameter of each zone of inhibition was measured in millimeter. The results were interpreted using a chart and recorded (CLSI, 2018).

Determination of Minimum Inhibitory Concentration of Acridine Orange

This was done according to the method described by Shuaibu *et al.* (2016). Each resistant bacterium was grown in nutrient broth. The minimum inhibitory concentration of the acridine orange used against the isolates was determined using a two-fold serial dilution technique. A stock solution of acridine orange (800µg/ml) was prepared. Seven test tubes containing 5 ml of nutrient broth were serially arranged. A serial dilution was carried out to give a concentration of 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25 µg/ml respectively. A 0.1ml volume of a standardized overnight culture of resistant isolates (inoculum size of 10cfu/ml) was then inoculated into each test tube and incubated at 37°C for 24 hours. The minimum concentration of acridine orange in which the isolates were susceptible was taken as the minimum inhibitory concentration of acridine orange.

Plasmid Curing Analysis

The curing (elimination) of the resistant plasmids of the bacteria isolate was done using a sub-inhibitory concentration of acridine orange, as described above. Isolates were subcultured for 24 hours at 37°C in nutrient agar plates containing the solution of acridine orange to cure the isolates (Shuaibu *et al.*, 2016).

Testing of the Cured Bacteria for Sensitivity against the Cured Plasmid

The cured bacteria were subjected to a sensitivity test against the same Beta-lactam antibiotics to which they were previously resistant to determine if the resistant plasmid was cured (Shuaibu *et al.*, 2016). The cured colonies were inoculated onto prepared Mueller Hinton agar plates. Then, antibiotic discs of

prior resistance were aseptically introduced into the plates, ensuring that the disc made appropriate contact with the surface of the agar. This was incubated for 24 hours at 37°C, after which plates were examined.

RESULTS

A total of one hundred (100) throat swab samples were collected from patients with upper respiratory tract infections in specialist hospital Sokoto. The bacteria identified and their frequency of occurrence is shown in Table 1. *Staphylococcus aureus* has the highest frequency of occurrence of 20 (57.2%), while the other two bacteria, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, have 9 (25.7%) and 6 (17.1%), respectively

Table 2 Presents the Minimum Inhibitory Concentration of Acridine Orange against the

isolates. All the isolates were susceptible at a Minimum Inhibitory Concentration of 400µg/ml Acridine Orange.

The antibacterial susceptibility test of the isolates before and after curing is presented in Table 3. *Staphylococcus aureus* resists Ceftazidime, Cefuroxime, Cloxacillin, Augmentin, and Cefoxitin. Only 4 (20%) are sensitive to Ceftriaxone. *Streptococcus pyogenes* are resistant to Ceftazidime, Cefuroxime, Cloxacillin, and Augmentin. Only 3 (33.3%) are sensitive to Ceftriaxone, and 2 (22.2%) are sensitive to Cefoxitin. *Pseudomonas aeruginosa* is resistant to Ceftazidime, Cefuroxime, Cloxacillin and Augmentin. Only 1 (16.7%) are sensitive to Ceftriaxone and Cefoxitin. All the isolates show reversion after plasmid curing by being susceptible to almost all antibiotics tested except for two antibiotics (Cloxacillin and Augmentin), in which all the bacteria maintained resistance.

Table 1: Frequency of Occurrence of the Bacterial Isolates

Organisms identified	Frequency of occurrence	Percentage (%)
<i>Staphylococcus aureus</i>	20	57.2
<i>Streptococcus pyogenes</i>	9	25.7
<i>Pseudomonas aeruginosa</i>	6	17.1
Total	35	100

Table 2: Minimum Inhibitory Concentration of Acridine Orange against the Isolates.

Concentrations (µg/ml)	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>Ps. aeruginosa</i>
400	+	+	+
200	-	-	-
100	-	-	-
50	-	-	-
25	-	-	-
12.5	-	-	-
6.25	-	-	-

Key : - = Not susceptible + = Susceptible

Table 3: Percentage sensitivity of test Bacteria to various Antibiotics before and after Curing.

Antibiotics	Sensitivity before curing			Sensitivity after curing		
	<i>S. aureus</i> (n=20)	<i>S. pyogenes</i> (n=9)	<i>Ps. aeruginosa</i> (n=6)	<i>S. aureus</i> (n=20)	<i>S. pyogenes</i> (n=9)	<i>Ps. aeruginosa</i> (n=6)
CAZ	100 R	100 R	100 R	25 S	66.7 S	50 S
CRZ	100 R	100 R	100 R	25 S	66.7 S	50 S
CTR	20 S	33.3S	16.7S	25 S	66.7 S	50 S
CXC	100 R	100 R	100 R	100 R	100 R	100 R
AUG	100 R	100 R	100 R	100 R	100 R	100 R
FOX	100 R	22.2 S	16.7S	25 S	66.7 S	50 S

Key: CAZ= Cefotaxime, CRX= Cefuroxime, CTR= Ceftriaxone, CXC= Cloxacillin, AUG= Augmentin, FOX= Cefoxitin, mm= millimetre, R=Resistance and S=Sensitive.

DISCUSSION

Staphylococcus aureus, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* were isolated and identified from samples. *Staphylococcus aureus* has the highest frequency of occurrence among the organisms isolated. The upper respiratory hosts many commensals and pathogenic bacteria, forming a complex microbial community. This community is assumed to be constantly subjected to synergistic and competitive interspecies interaction (Astrid *et al.*, 2016). The bacteria isolated can be transferred to healthy and immunocompromised individuals interacting with the infected subjects (persons). These findings agree with Atata *et al.* (2013), who isolated similar organisms and reported that *Staphylococcus aureus* has the highest frequency of occurrence.

The antimicrobial susceptibility test of the isolates before plasmid curing showed that all the isolates were sensitive to Ceftriaxone. However, *Staphylococcus aureus* resists Cefotaxime, Cefuroxime, Cloxacillin, Augmentin, and Cefoxitin. In addition to ceftriaxone susceptibility, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* were also sensitive to Cefoxitin. All the bacteria resisted Cloxacillin and Augmentin before and after plasmid curing. Most bacterial pathogens

causing upper respiratory tract infections have a number of virulence factors, including the production of betalactamases and the exchange of resistance markers. The high rate of resistance of the bacterial isolates to the antibiotics might be due to the widespread use of antimicrobial agents in the hospital, the ease of availability, and indiscriminate use of these drugs within and outside the Hospital, as many of the drugs are available over the counter for self-medication. Resistance to antimicrobial agents is a problem in communities and hospitals, but in hospitals, transmission of bacteria is amplified because of the presence of a highly susceptible population. Reasons that could be associated with the transmission of resistant strains of these microorganisms include poor hygiene, overcrowding, absence of an effective infection control program, and deficiency of trained infection control workers.

Drug resistance property of bacteria is usually found in R-plasmid, which can be disseminated to diverse populations and regions causing worldwide problems (Taura, 2021). This resistance pattern agrees with Shuaibu *et al.* (2016) and Atata *et al.* (2013), who found that most isolates from clinical samples had maximum resistance to beta-lactam antibiotics. The sensitivity of test bacteria to various antibiotics after curing showed that all the isolates were susceptible to almost all

antibiotics tested except Cloxacillin and Augmentin, to which all bacteria maintained resistance. The continuous resistance of some of the isolates to some of the antibiotics even after plasmid curing implies that the resistance genes are not plasmid-mediated and might be a result of intrinsic factors, environmental factors, such as indiscriminate use of antibiotics, emergence of new strains of the organisms among others. These are supported by the findings of Shuaibu *et al.* (2016), who also reported that the isolates were resistant to cloxacillin and Augmentin before and after plasmid curing.

CONCLUSION

This study identified *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* as the bacterial isolates from patients with upper respiratory tract infections attending specialist hospital Sokoto. Before plasmid curing, these isolates resisted Ceftazidime, Cefuroxime, Cloxacillin, and Augmentin. After plasmid curing using acridine orange (400µg/ml), All the isolates reverted to being susceptible to almost all antibiotics tested except Augmentin and Cloxacillin, which were resisted by all the bacteria.

RECOMMENDATIONS

1. Encouraged antimicrobial susceptibility against upper respiratory tract pathogens on a routine basis to figure out the best antibiotic for treating respiratory tract infections.
2. Extensive use and misuse of antimicrobial drugs should be avoided, which will reduce the emergence of drug resistance. There is a need to control bacterial contamination and improve hygienic conditions, especially in rural areas where health problems are major issues.

REFERENCES

- Astrid, B. A., Levin, E., Van Houten, M. A., Hasrat, R., Kalkman, G., Biesbroek, G. and Bogaert, D. (2016). Development of upper respiratory tract microbiota in infancy is affected by mode of delivery. *EBioMedicine*; **9**:336-345. [[Crossref](#)]
- Atata, R. F., Ibrahim, Y. K., Giwa, A. and Akanbi, A. A. (2013). Antibiotics of Bacterial Isolates from surgical site and hospital environment in a university teaching Hospital in Nigeria. *Journal of Medicine and Medical Science*; **4**: 181-187. [[Crossref](#)]
- Clinical and Laboratory Standards Institute. 2018. *Performance standards for antimicrobial susceptibility testing; 27th informational supplement. M100-528*. Clinical and Laboratory Standards Institute, Wayne, PA.
- Gambo, S., Mohammad, U-K., Baki A.S., Bello, A. and Gambo, A. (2017). Incidence of Salmonella species isolated from presumptive Typhoid fever patients attending Specialist Hospital Sokoto. *Scholarly Journal of Biological Science*; **6**(2):23-27.
- Kho, B. P., Ong, C. M, Tan, F. T. and Wee, C. Y. (2013). Antibiotic prescribing for upper respiratory tract infections in sarawak district hospitals. *The Medical Journal of Malaysia*; **68**: 136-40. <https://europepmc.org/article/med/23629559>
- Kutter, J. S., Spronken, M. I., Fraaij, P. L., Fouchier, R. A. and Herfst, S. (2018). Transmission routes of respiratory viruses among humans. *Current opinion in virology*, **28**, 142-151. [[Crossref](#)]
- Mawaddah, A., Genden, H. S., Lum, S. G. and Marina, M. B. (2020). Upper respiratory tract sampling in COVID-19. *The Malaysian journal of pathology*; **42**(1), 23-35. <http://www.mjpath.org.my/2020/v42n1/sampling-in-COVID-19.pdf>
- Miriti, D. M., Muthini, J. M. and Nyamache, A. K. (2023). Study of bacterial respiratory infections and antimicrobial susceptibility profile among antibiotics naive outpatients visiting Meru teaching and referral hospital, Meru County, Kenya in 2018. *BMC Microbiology*; **23**(1):172. [[Crossref](#)].
- Patwardhan, R. B., Dhakephalkar, P. K., Chopade, B. A., Dhavale, D. D. and Bhonde, R. R. (2018). Purification and Characterization of an Active Principle, Lawsone, Responsible for the Plasmid Curing Activity of *Plumbago zeylanica* Root Extracts. *Front Microbiol*; **9**:2618. [[Crossref](#)].
- Peroš-Golubičić, T. and Tekavec-Trkanjec, J. (2015). Upper respiratory tract infections. *Textbook of Respiratory and Critical Care Infections. New Delhi: Jaypee Brothers*, 16-29. [[Crossref](#)]
- Shuaibu, A. S., Ibrahim, Y. K., Olayinka, B. O., Atata, R. F., Ungo-kore, H. Y.,

Olowookere, A. and Shuaibu A. B. (2016). Antiplasmid effect of acridine orange on multiple antibiotics resistant bacteria isolated from a secondary health care institution in Sokoto state. *Journal of pharmaceutical and allied sciences*; 13:2-7.

Taura, D. W. (2021). Bacterial isolates of the respiratory tract infection and their current sensitivity pattern among patients attending Aminu Kano Teaching Hospital Kano, Nigeria. *International Journal of Biomedical and Health Sciences*: 7(1):45-51. [[Crossref](#)]