Original Research Article

Prevalence and Antibiogram of *Rothia mucilaginosa* and *Staphylococcus* spp Isolated from Oral Cavity of Students in a Tertiary Institution

ABSTRACT

Bacteremia and systemic infection caused by *Rothia mucilaginosa* and *Staphylococcus* spp are uncommon and their resistance to antibiotics are becoming overwhelming. Hence, this study was carried out to investigate the prevalence and antibiogram of *Rothia mucilaginosa* and *Staphylococcus* spp from oral cavity of students in a Tertiary Institution**.** A total of fifty (50) specimen were collected from the oral cavity of male and female students in Rivers State University and subjected to standard microbiological procedures such as culturing, isolation, identification as well as antibiotic susceptibility test. Sixty-seven (67) isolates were identified where *Staphylococcus aureus* were 43 isolates, *Rothia mucilaginosa* were 18 isolates and six (6) isolates were *Staphylococcus epidermidis.* All the isolates of *Rothia mucilaginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* were positive for the presence of haemolysin and catalase enzymes. The results of the percentage occurrence among male and female students revealed that *Staphylococcus aureus* had the highest occurrence (64.18%:59.38%) followed closely by *Rothia mucilaginosa* (22.86%:31.25%) while *Staphylococcus epidermidis* had the least prevalence (8.95%:9.37%) in male and female respectively. The result of the susceptibility patterns showed that majority of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Rothia mucilaginosa* were highly sensitive to ciprofloxacin (90.70%; 100%; 83.33%) and gentamicin (76.74%; 83.33%: 100%), and highly resistant to azithromycin (44.19% and 66.67%) for *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Rothia mucilaginosa* were highly resistant to ampiclox (61.11%). The results of the multidrug resistance index of 37 (86.05%) of the 43 *S. aureus* isolates had a MAR index ≥ 0.2, 15 (83.33%) of the 18 *R. mucilaginosa* isolates had MAR index ≥ 0.2 and (100%) of the *S. epidermidis* also had a MAR index ≥ 0.2. This study displays about serious threat to public health because these microorganisms can become pathogen causing diseases such as septicemia, endocarditis, and other life-threatening infections. It also illustrated the risks associated with the indiscriminate use of antibiotics are advised.

Keywords: Antibigram, Prevalence, *Rothia mucilaginosa*, Oral cavity and Multiple Antibiotic Resistance (MAR) Index

INTRODUCTION

Rothia mucilaginosa (formerly known as *Stomatococcus mucilaginosus*) is a Gram-positive, coagulase-negative, encapsulated, non-spore-forming and non-motile coccus, present in clusters, tetrads or pairs that is a part of the normal oropharyngeal flora (a normal resident of the human mouth and nasopharynx). Belonging to the family of Micrococcaceae, it was first isolated from the mucous membrane of the cheek and gingiva. It is an oral commensal, that has been linked to causing severe bacteremia in immunocompromised patients. This bacterium has also been shown to form biofilms, similar to that of *Pseudomonas aeruginosa* and some *Staphylococcus* species. *R. mucilaginosa* is a cohabitant in the lower airways of patient with bronchiectasis (Fanourgiakis *et al.*, 2003; Maraki and Papadakis, 2015).

R. mucilaginosa is a normal inhabitant of the human oral cavity and respiratory tract. It is an infrequent pathogen, mostly affecting severely immunocompromised patients. About 70

cases of bacteremia, endocarditis, catheter-associated bloodstream infection, central nervous system infections, endophthalmitis, spondylodiscitis, osteomyelitis, prosthetic joint infection, pneumonia, cholangitis and peritonitis have been described (Almuzara *et al*., 2004).

On culture, it usually appears as gram-positive cocci in clusters-hence, the previous classification as a *Stomatococcus. R. mucilaginosa* is a rare cause of true bacteremia and sepsis; two case series from large academic institutions each identified more than 20 patients with true *Rothia bacteremia* (Ochi *et al*., 2017). *R. mucilaginosa* was identified as the predominant species recovered; the majority of patients had neutropenia and hematologic malignancy. *R. mucilaginosa* has been found in cases of pneumonia in patients with leukemia and lung cancer and peritonitis in patients undergoing a case of granulomatous dermatitis attributable to *R. mucilaginosa* bacteremia that has been reported (Ochi *et al*., 2017; Rigauts *et al*., 2022).

Staphylococcus is a genus of Gram-positive bacteria in the family Staphylococcaceae from the order Bacillales and can also be found in the mucous membrane (Tong *et al*., 2015). Under the microscope, they appear spherical (cocci), and form in grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms (capable of growth both aerobically and anaerobically). *Staphylococcus* was one of the leading infections in hospitals and many strains of this bacterium have become antibiotic resistant. Despite strong attempts to get rid of them, staph bacteria stay present in hospitals, where they can infect people who are most at risk of infection (Tong *et al*., 2015).

Excessive antibiotic usage is thought to be the primary cause of antibiotic resistance. It is possible for this antibiotic resistance to develop due to gene mutations or horizontal gene transfer of this organism, *R. mucilaginosa* as well as *Staphylococcus* species (Laxminarayan *et al*., 2001). Hence, this study was carried out to investigate the prevalence and antibiogram of *Rothia mucilaginosa* and *Staphylococcus* spp from oral cavity of students in a tertiary institution.

MATERIALS AND METHODS

Description of the study area

This study was carried out in different faculties in Rivers State University, Nkpolu-Oroworukwo.

Collection of Specimen

A total of fifty (50) oral swab specimen were collected from the oral cavity of students in Rivers State University under aseptic conditions and immediately transported to the Department of Microbiology laboratory for bacteriological analysis.

Microbiological Analysis

The swab specimen was inoculated onto prepared sterile blood/chocolate agar and mannitol salt agar plates using streak plate method, properly labelled and incubated at 37^0C for 24 hours.

Characterization and Isolation of the Test Organism

Discreet colonies were obtained from the plates, characterized morphologically based on colony size, shape, pigmentation, elevation and texture and sub-cultured onto prepared nutrient agar plates and incubated at 37^oC, for 24hours to obtain pure cultures of the organisms and further characterized microscopically and gram stained (Taylor, 2008).

Preservation and Identification of the Bacterial Isolates

The pure cultures were stored in sterile 10% (v/v) glycerol suspension at -4^oC as a cryopreservative agent to prevent the damage of the pure cultures during drying for further analysis. Identification of the organism were further carried out through series of biochemical tests and virulent test such as Oxidase, Catalase, Coagulase, Citrate Utilization, motility, Methyl red, Indole, Voges Proskauer, sugar fermentation tests and hemolysis test to confirm the test organisms (Cheesbrough, 2006).

Antibiotic susceptibility testing

The antimicrobial susceptibility profiles or pattern of the bacterial isolates to antibiotics were determined using the Kirby-Bauer disk diffusion method on sterile Mueller-Hinton agar. Standardization of the bacterial isolates in a suspension was carried out by adjusting to 0.5 McFarland turbidity standards containing $x10^8$ cells. A sterile swab was dipped into the bacteria suspension, pressed on the side of the test tubes to allow excess drip off and then used to evenly streak the entire surface of the Mueller Hinton agar and rotating the agar plate 60º each time to ensure even distribution of the inoculum (CLSI, 2017). The plates were left to air dry for 3–5 min. Conventional antibiotics disk impregnated with Ampiclox (20mg), Amoxil (20mg), Rifampin (20mg), Ciprofloxacin (10mg), Streptomycin

(30mg), Septrin (30mg), Erythromycin (30mg), Pefloxacin (10mg) and Gentamicin (10mg) were aseptically placed on the surface of the inoculated agar plate with sterile forceps. Each disk was pressed down to make full contact with the surface of the agar. The plates were then incubated for 24 hours at 33 to 35ºC in an inverted position. The zones of inhibition were measured in millimetre (mm) using a meter rule and compared to (CLSI, 2017).

Determination of Multiple Antibiotic Resistance (MAR) indexes

Multiple antibiotic resistance is the resistance of bacterial isolates to three or more antibiotics (Osundiya *et al*., 2013). Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula MAR = a/b, where ''a'' represent the number of antibiotics to which the test isolates depicted resistance and ''b'' represent the total number of antibiotics to which the test isolate has been tested for susceptibility (Krumperman, 1985).

Data analysis

Statistical Package for Social Sciences (SPPSS) version 22 was used to analyse the data obtained from the measurement of the zones of inhibition. Descriptive statistics were used to summarize all data obtained (Bewick *et al*., 2004).

RESULTS

Results of the morphological and biochemical characteristics of the bacterial isolates is shown in Table 1. A total of sixty-seven (67) isolates were identified as *Staphylococcus aureus, Rothia mucilaginosa* and *Staphylococcus* epidermidis, forty-three (43) *Staphylococcus aureus,* eighty (18) *Rothia* mucilaginosa and six (6) *Staphylococcus epidermidis* from the fifty (50) swab specimen collected from male (25) and female (25) within the University.

Generally, the results of the abundance of the bacterial isolates among male and female students analyzed shown that *Staphylococcus aureus* had the highest prevalence with 64.18% and *Staphylococcus epidermidis* had the least with 8.95% as shown in Fig. 1. Also, the distribution of bacterial isolates among male and female students shown *Staphylococcus aureus* had the highest percentage occurrence among male and female students with 68.57% and 59.38% while *Staphylococcus epidermidis* had 8.57% and 9.37% respectively as shown in Fig. 2.

Results of the virulence properties revealed that all the isolates were positive for haemolysin and catalase enzymes (Table 1).

The results of the susceptibility pattern of bacterial isolates as shown in Table 2, majority of *Staphylococcus aureus* isolates were highly sensitive to ciprofloxacin (90.70%), followed by pefloxacin (79.07%), gentamycin (76.74%), septrin (74.42%), and amoxil, rifampicin (72.09%) and more resistant to Azithromycin (44.19%) and ampiclox (41.86%). *Staphylococcus epidermidis* sensitive to ciprofloxacin (100%), streptomycin, septrin and rifampicin (83.33%) while *Rothia mucilaginosa*, it was highly resistant to ampiclox (83.33%) and (61.11%), but highly sensitive to gentamicin (100%), ciprofloxacin (88.89%) and rifampicin (88.89%). The results of multidrug resistant index are shown in Table 3. The multidrug resistance index of 37 (86.05%) of the 43 *S. aureus* isolates had a MAR index greater than 0.2, 15 (83.33%) of the 18 *R. mucilaginosa* isolates had MAR index greater than 0.2 and (100%) of the *S. epidermidis* also had a MAR index greater than 0.2

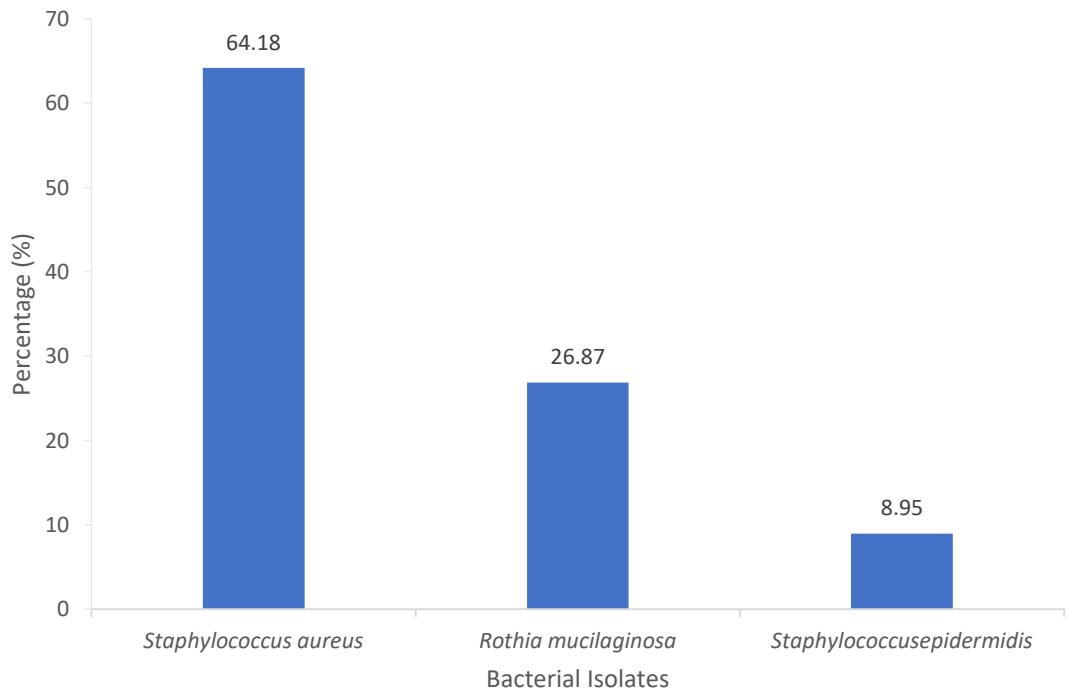

Fig. 1: Percentage occurrence of the bacterial isolates

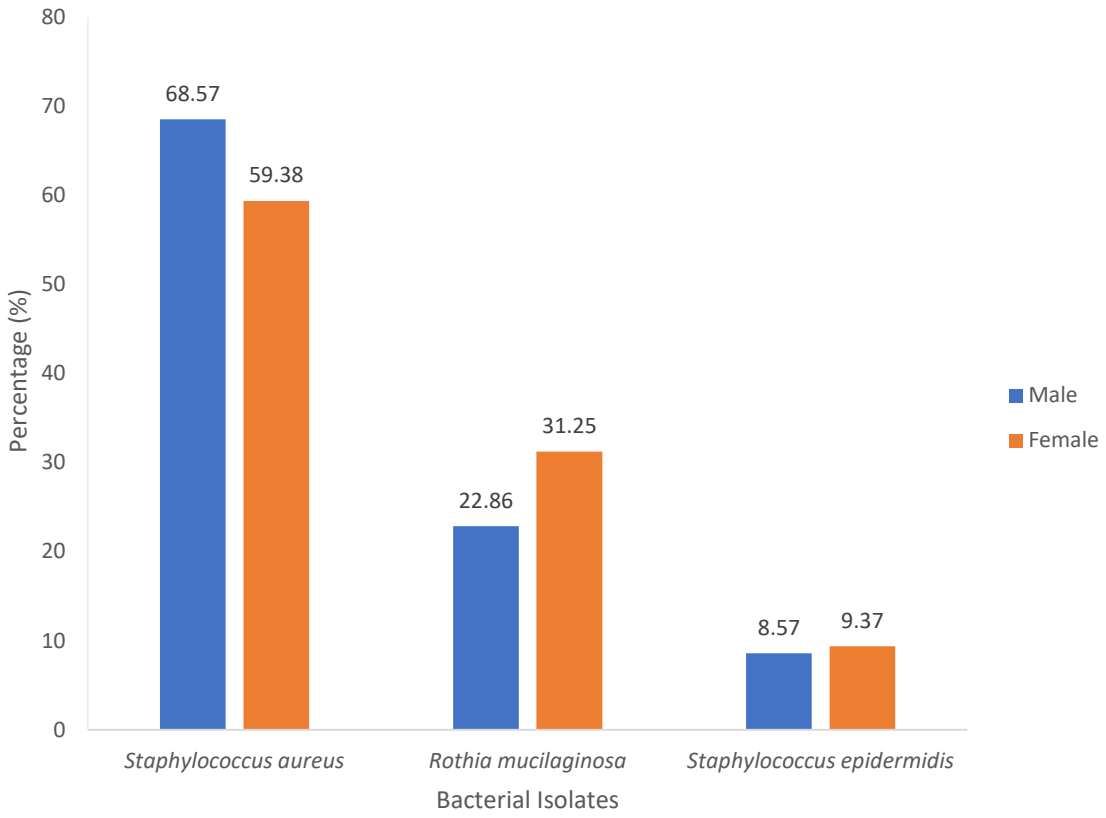

Fig. 2: Distribution of Bacterial Isolates Among Male and Female Students

Table 1: Virulence Characterization of Bacterial Isolates

Probable Organism	Catalase	Oxidase	Hemolysis	Coagulase
Staphylococcus aureus	+	-	α	+
Rothia mucilaginosa	+	-	α	-
Staphylococcus epidermidis	+	-	α	-

Key: Positive (+), Negative (-), α-alpha-haemolysis

Table 2: Susceptibility Patterns of Bacterial Isolates

Antibiotics	***Staphylococcus aureus*** **N=43**			***Staphylococcus epidermidis*** **N = 6**			***Rothia mucilaginosa*** **N = 18**		
	R n (%)	**I n (%)**	**S n (%)**	**R n (%)**	**I n (%)**	**S n (%)**	**R n (%)**	**I n (%)**	**S n (%)**
Ampiclox-20mg	18(41.86)	0(0.00)	25(58.14)	5(83.36)	0(0.00)	1(16.67)	11(61.11)	0(0.00)	7(38.89)
Azithromycin 15mg	19(44.19)	0(0.00)	24(55.81)	4(66.67)	0(0.00)	2(33.33)	7(38.89)	0(0.00)	11(61.11)
Amoxil 20mg	12(27.91)	0(0.00)	31(72.09)	2(33.33)	0(0.00)	4(66.67)	3(16.67)	0(0.00)	15(83.33)
Rifampicin 20mg	12(27.91)	0(0.00)	31(72.09)	1(16.67)	0(0.00)	5(83.33)	2(11.11)	0(0.00)	16(88.89)
Ciprofloxacin 10mg	4(9.30)	0(0.00)	39(90.70)	0(0.00)	0(0.00)	6(10.0)	2(11.11)	0(0.00)	16(88.89)
Streptomycin 30mg	14(32.56)	0(0.00)	29(67.44)	1(16.67)	0(0.00)	5(83.33)	4(22.22)	0(0.00)	14(77.78)
Septrin 30mg	11(25.58)	0(0.00)	32(74.42)	1(16.67)	0(0.00)	5(83.33)	7(38.98)	0(0.00)	11(61.11)
Erythromycin 30mg	17(39.53)	0(0.00)	25(58.14)	2(33.33)	0(0.00)	4(66.67)	7(38.98)	0(0.00)	11(61.11)
Pefloxacin 10mg	9(20.93)	0(0.00)	34(79.07)	2(33.33)	0(0.00)	4(66.67)	6(33.33)	1(5.56)	11(61.11)
Gentamycin 10mg	10(23.26)	0(0.00)	33(76.74)	1(16.67)	0(0.00)	5(83.33)	0(0.00)	0(0.00)	18(1.00)

KEY: R (Resistant), I (Intermediate), S (Susceptibility)

Table 3: MAR Index of Bacterial Isolates from all the Samples analyzed

MAR Index	*Staphylococcus aureus* N = 43	*Staphylococcus epidermidis* N = 6	*Rothia mucilaginosa* N =18
	n (%)	**n (%)**	**n (%)**
0.0	3(6.98)	0(0.00)	1(5.56)
0.1	3(6.98)	0(0.00)	2(11.11)
0.2	10(23.26)	1(16.67)	5(27.78)
0.3	11(25.58)	3(50.00)	3(16.67)
0.4	9(20.93)	2(33.33)	7(38.88)
0.5	4(9.30)	0(0.00)	0(0.00)
0.6	2(4.65)	0(0.00)	0(0.00)
0.7	0(0.00)	0(0.00)	0(0.00)
0.8	0(0.00)	0(0.00)	0(0.00)
0.9	0(0.00)	0(0.00)	0(0.00)
1.0	1(2.32)	0(0.00)	0(0.00)
	MAR Index >0.2 86.05%	**MAR Index>0.2 100%**	**MAR Index>0.2 83.33%**

KEY: Multiple Antibiotic Resistance (MAR)

DISCUSSION

Antibiotic resistance of *R. mucilaginosa S. aureus* and *S. epidermidis* has been a problem worldwide and *R. mucilaginosa* is evolving new ways to resist antibiotics, which is a serious public health concern (Emmanuel and Magaji, 2011). Significant number of *R. mucilaginosa S. aureus* and *S. epidermidis* were isolated from the oral cavity from both male and female. The prevalence of *Staphylococcus aureus* was high male compared to their female counterpart and this is because of the poor oral hygiene of the individual sampled. *Rothia mucilaginosa* and *Staphylococcus epidermidis* were more in the females than males. Personal hygiene deficiency has been the major reasons why *Staphylococcus epidermidis* and *Staphylococcus aureus* are predominant (Nurain *et al*., 2015). This can also be associated with the poor ambient air quality of the environments (Klem-patel *et al*., 2006). Though work has not been done on their distribution in male and female. The findings also showed that *Rothia mucilaginosa* is a common pathogen in the oral cavity in human, causing bacterial infections, particularly endocarditis (Tsuzukibashi *et al.*, 2017; Robertson *et al.*, 2021). This finding is comparable to that of Amir *et al.*, (2020), who also found a high prevalence of *Staphylococcus aureus* and *Rothia mucilaginosa* in oral cavity at 40% from clinical samples. They were the most prevalent commensal inhabitant of the gastrointestinal tract, it is a common pathogen liked with community-associated as well as hospital-acquired infections

(Nurain *et al*., 2015). Several other studies confirming similar results (Nurain *et al*., 2015; Sharma *et al*., 2011)

The susceptibility pattern showed that a significant number of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Rothia mucilaginosa* isolates were highly sensitive to ciprofloxacin, pefloxacin, gentamicin, septrin and amoxil, rifampicin. *Staphylococcus epidermidis* and *Rothia mucilaginosa*, it was highly resistant to ampiclox and azithromycin. The antibiotic sensitivity patterns of *R. mucilaginosa, S. aureus* and *S. epidermidis* found in this study has significant impacts on the public health implications of these organisms because it affects the clinical treatment option(s) that are accessible for therapy (Emmanuel and Magaji, 2011). The sensitivity of organisms to antimicrobials put organisms under selective pressure, which is a major problem in epidemiological investigations. The results of the antibiotic sensitivity pattern are in line with the work of Emmanuel and Magaji (2011), which showed that *Staphylococcus aureus* is most sensitive to gentamicin, ciprofloxacin, streptomycin and chloramphenicol isolated from clinical samples.

Higher percentages of the *Rothia mucilaginosa*, *Staphylococcus aureus* and *S. epidermidis* had a higher multidrug resistant index. Today development of multidrug resistance is become natural phenomenon, due to interestingly raise in the number of immunocompromised conditions, blind and improper use of broad spectrum of antibiotics as well as poor infection prevention, beside that patients profile, environmental and geographical factor were among important player determining the bacterial profile and resistance pattern (Lebea and Davies, 2017; Weinstein, 2001). The existence of multi-drug resistant strains demonstrates how *R. mucilaginosa* is generating these new strategies, which are limiting and expensive therapeutic choices (Davis *et al*., 2016).

Conclusions and Recommendations

The high prevalence of *R. mucilaginosa, S. aureus* and *S. epidermidis* in oral cavity is a serious threat to public health because they can become a pathogen causing diseases such as septicemia, endocarditis, and other life threatening infections. This study has confirmed that *R. mucilaginosa, S. aureus* and *S. epidermidis* were resistant to several classes of antibiotics but gentamicin, ciprofloxacin, rifampicin, amoxil and Streptomycin can be used as first-line medications for treatment of *R. mucilaginosa*-related oral cavities as well as for *S. aureus* and *S. epidermidis* infections. Surveillance systems should be increased for assessing risk factors of diseases and to provide strategies to prevent and protect *R. mucilaginosa* infections and to

stop the spread of antibiotic resistance strains as well as public awareness campaigns about the risks associated with the indiscriminate use of antibiotics are advised.

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