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Prevalence of Meticillin Resistance *Staphylococcus Aureus* in Patients with Lower Respiratory Tract Infections

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ABSTRACT

Background: Lower respiratory tract infection (LRTIs) is an emerging and threatening disease of both adults and children. Methicillin resistant *Staphylococcus aureus* (MRSA) remains a life threatening pathogens that may colonize the lungs and cause Staphylococcal related lower respiratory tract infection. The aim of this study was to determine the prevalence and risk factors associated with MRSA infection among individuals with lower respiratory tract infections in a community setting.

Method: A structured questionnaire consisting of demographic characteristics, antibiotic usage, social life habits were administered to 152 participants, comprising of 80 males and 72 females. The subjects were selected based on non- hospitalization for the past six months. Sputum samples were collected and cultured unto Mannitol Salt agar. The *Staphylococcus aureus* isolates were sub-cultured unto Chromagar MRSA for MRSA identification. The isolates were subjected to antibiotic susceptibility testing, using disc diffusion method.

Result: The MRSA were highly resistant to Ampiclox (96.2%), Amoxcillin (92.3%), Rifampicin and Streptomycin (80.8%), Norfloxacin (76.9%), Erythromycin and Chroramphenicol (69.2%), and Levofloxacin (65.4%).

Conclusion: This study showed that MRSA is implicated in LRTIs and is brought about by improper therapy and lack of awareness.

KEYWORDS: Methicillin resistant *Staphylococcus aureus*, Respiratory tract infection.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a multi-drug resistant strains of *Staphylococcus aureus*, resistant to penicillin and cephalosporins.[1]. There are two main classes of MRSA, Community Associated-Methicillin resistant Staphylococcus aureus (CA-MRSA) and Health-care Associated-Methicillin resistant *Staphylococcus aureus* (HA-MRSA). The two main classes apart from showing their genotypic differences, also show the epidemiological and clinical features of the infections they cause.[2] Any infection with MRSA isolates occurring among out-patients or in-patients after 48 hours of hospitalization are considered CA-MRSA Infection.

CA-MRSA has its onset in the community in an individual lacking the knowledge of MRSA risk factors while HA-MRSA has its onset with healthcare associated risk factors such as hospitalization, chronic dialysis, antibiotic treatment or exposure to invasive procedures.[3] HA-MRSA strains that occur in the health sector can also be seen in the community, the CDC named a third category called Healthcare-Associated Community Onset MRSA" (HACO-MRSA) infection.[4]

The pathogenicity of *Staphylococcus aureus* infection is associated with various bacterial surface components such as Capsular polysaccharide and Protein A, Clumping factor and Fibronectins, binding protein and extracellular proteins like coagulase, haemolysins, enterotoxin, toxic shock syndrome toxins and exfoliatins. MRSA being a strain of *Staphylococcus aureus* are likely to have one or more of these pathogenicity traits.[4,5] MRSA emerged as a nosocomial pathogen in the early 1960s. Hospital outbreaks have been reported in USA and Europe. MRSA infections have been acquired almost exclusively in hospitals, long term care facilities or similar institutional settings.[6] Tong in his work.[7] gave an account of the study in Houston which showed that CA-MRSA accounted for 56% in 2001, 57% in 2002, 78% in 2003 and recorded a prevalence of 42% in a village in the Western Australia. Community -associated MRSA infection can pose hazardous effect on health but the major concern is that, it may have

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devastating consequences, if it becomes epidemic in resource poor regions.[8] HA-MRSA is a highly resistant nosocomial pathogen in both acute and chronic infections. It is associated with increased morbidity and mortality when compared with CA-MRSA.[9] Risk factors for MRSA infections in the hospital include prior antibiotic exposure, staying on admission in the hospital, surgery and exposure to MRSA colonized patients.[10] Humans are a natural reservoir for *Staphylococcus aureus* and asymptomatic colonization is very common. Colonization of the nasopharynx, perineum or non-intact skin, may occur shortly after birth and may reoccur anytime thereafter.[10] Young children tend to have higher colonization rates, probably because of their frequent contact with respiratory secretions. [11] The initial presentations of MRSA infection are small red bumps that resemble spider bites, pimples, boils that may be accompanied by fever and occasionally rashes which become larger, more painful and eventually open to deep pus filled boils.[12] HA-MRSA infections may include surgical injuries, urinary tract infections, bloodstream infections and pneumonia.[13].The most common manifestations of CA-MRSA are skin infections such as necrotizing fasciitis or pyomyositis, necrotizing pneumonia, infective endocarditis, bone or joint infections.[14]

MRSA causes varied infections, which may progress substantially within 24-48 hours and within few days MRSA can take hold of human organs, tissues and bones in the body and it's resistance to certain antibiotic makes it very difficult to treat and it can be a great risk to health, hence this work is aimed at determining the association of MRSA in lower respiratory tract infections and survey the antibiotic resistance patterns of the MRSA isolates.

MATERIALS AND METHODS

Study population: The subjects presenting with lower respiratory tract infections based on persistent cough and X-ray indicating pneumonia were recruited for the study. They were people that have not been hospitalized for the previous 6 months. Information on their demographic and health status was abstracted using a questionnaire or by oral means from the patient. The subjects were recruited from the community population at Oko and Ekwulobia Town, Anambra State, Eastern-Nigeria.

Cultural methods: Early morning sputa were collected in wide mouth containers by subjects, based on instructions to collect early morning deep cough from the lungs. These were aseptically processed using standard methods. Identification of *Staphylococcus aureus* by the classical method of gram reaction, catalase and coagulase tests were carried out. The *Staphylococcus aureus* isolates were sub-cultured unto Chromagar MRSA (Rambach-Agar) and were confirmed by their particular growth patterns. Colonies of Methicillin resistant *Staphylococcus aureus* were identified as mauve/pink colonies on Chromagar Mrsa agar and by their resistance to Naficillin, Oxacillin and Penicillin.

Antibiotic Susceptibility Test: The antibiotics used for the susceptibility test on the MRSA isolates were produced by Oxoid Biologicals. They are: Ciprofloxacin (10mcg), Norfloxacin(10mcg), Gentamycin(10mcg), Amoxicillin(20mcg), Streptomycin(30mcg), Erythromycin(30mcg), Chloramphenicol(30mcg), Rifampicin(20mcg), Ampiclox(20mcg), Levofloxacin(20mcg). The antibiotic susceptibility testing was done by disc diffusion method in accordance with Clinical and laboratory Standard Institute (CSLI) guideline using the discs mentioned above. The organism was grown on peptone water between 4-6 hours. The culture broth was flooded unto nutrient agar and the excess decanted, and then the various discs were placed on the plate equidistant from each other. It was incubated at 37℃ for 24 hours and the zones of inhibition measured with a ruler and it was further compared with the standard values.

Statistical Analysis: Data obtained was analyzed using SPSS package.

Chi square was used to determine the effect of the demographic features of sex, age, occupation, co-morbid factors, duration of sickness, length of antibiotic usage, previous antibiotics used, a P-value of ≤ 0.05 was regarded as statistically significant.

RESULTS

Demographic Characteristics: A total of 152 sputa samples from individuals with of lower respiratory tract infections were analyzed. They comprised of 80(52.6%) males and 72 (47.4%) females, with mean age of 30.8 ±11.3 year-olds (median: 27.0 years) and age range of between 14 - 80 year-olds. The patients with *Staphylococcus aureus* isolates were 45 (29.6%), while 26 (17.1%) were colonized by methicillin resistant *Staphylococcus aureus* (MRSA). The males had higher MRSA infections than the females, 9.9%(15/152) and 7.2% (11/152) respectively. The age group difference was statistically significant for MRSA colonization of the lower respiratory tract system. The participants below 20 years old had no MRSA, whereas those of 21-40, 41-60 and above 60-

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year olds accounted for 11.8%, 3.9% and 1.3% respectively. The participants that were farmers and traders, had MRSA isolates of 3.9% and 4.6% respectively, while the students with MRSA isolates were 6.6% and civil servants were 1.9%; and these were statistically significant, it is shown in Table1.

Risk factors for MRSA Infection: Participants with previously identified illnesses (co-morbid factors) were highly infected with MRSA. Those with pulmonary pneumonia accounted for 5.9% while those with diabetes mellitus, HIV and high blood pressure had 1.3% each. Those without any co-morbid factors accounted for 8.6% of the MRSA isolates as shown in (Table1). The duration of sickness was analyzed based on oral interview with the subjects. The duration of sickness was statistically significant. The participants with more than five weeks of persistent cough had more MRSA isolates. The previous use of antibiotics showed that the 17.1% MRSA isolates were on antibiotics prescribed by their physician during out-patients department visits. This can be seen in (Table 2).

The social life habits of smoking cigarettes and alcohol consumption were not statistically significant for infections with MRSA(Table 2). Multi-variate analysis was carried out and it showed that sex, age, occupation, co-morbidity, duration of sickness, length of antibiotic use, previous antibiotics used, were co-factors for MRSA infection. P-value ≤ 0.05 (Table 3). MRSA isolates showed varying degrees of resistance with the highest resistance to Ampiclox(96.2%), while Amoxicillin had 92.3%, both Rifampicin and Streptomycin had 80.8%, Norfloxacin, 76.9%, Erythromycin and Chroramphenicol both had 69.23% and Levofloxacin,65.4%. The MRSA were more susceptible to Ciprofloxacin, 84.6% and Gentamycin, 65.4%. (Table 4).

DISCUSSION

The emergence of Methicillin resistant *Staphylococcus aureus* has modified the pathogenicity of *Staphylococcus aureus* giving it virulent ability to cause diverse array of life threatening infections and the capacity to adapt to different environmental conditions.[11] This study, showed a prevalence rate of 17.1% of MRSA in LRTIs, the subjects has never been hospitalized for the past six months this showed that acquiring the MRSA infection may be through person to person contact.[15] The occurrence of MRSA in Nigeria has been previously reported by,[16] They reported differing prevalence rates, from different clinical conditions and concluded that CA-MRSA is prevalent in Southern Nigeria. They also isolated and characterized MRSA from surgical and pediatrics patients. In this study, the studied subjects were unaware of their carrier status of MRSA and this may consequently result in the patients having necrotizing pneumonia.[17] or even leucopenia.[18]

The age difference among the participants was a major factor in acquiring MRSA infection. The majority of MRSA Isolates 11.8% were from the age group of 21-40 years old. The age prevalence agreed with,[19] they reported that those aged less than 35 years old were more predisposed to MRSA infection. Smoking of cigarettes and alcohol consumption may have enhanced the colonization of the lower respiratory tract by MRSA. This study, also show that prolonged use of antibiotics whether prescribed or un-prescribed by a physician may have enhanced the infection. This is consistent with drug usage in developing countries, where drug monitoring is not critical. In contrast,[20] agreed that in Denmark, the low consumption of antibiotics and the introduction of control measures brought about the decline of MRSA in *Staphylococcus aureus* bacteremia from approximately 20% in 1960s to less than 1% throughout the 1980s and 1990s. The bias in the use of antibiotics in the subjects was that majority accepted the drugs prescribed by a physician and also took some other antibiotics. This assumption agreed with,[21] which reported that consistent changes of antibiotics used for therapy can lead to toxicity and multi- drug resistance.

In analyzing the factors that may predispose an individual to MRSA infection, co-morbidity was statistically significant. Among the co-morbid factors, the existent of pulmonary pneumonia was evident.[22] The other factors that were significant are duration of infection, the longer the duration the more invasive the MRSA organism and the more spread. [18] The previous use of antibiotics for more than ten days accounted for higher MRSA infection as 10.5% of the patients that has used antibiotics were affected. This factor manifested in the resistant pattern of the organism to various antibiotics used, this agreed with[20] who identified prolonged exposures to antimicrobials agents as a risk factor. Naves in his work [23] concluded that changes in rates of resistance to other antibiotics among MRSA may reflect the prevalent epidemic strains of MRSA and this conclusion reflected in the present study, where some of the patients had used various antibiotics in self-medication before reporting to the hospital. The MRSA organisms were majorly resistant to Ampiclox, which is a penicillin derivative. The mechanism of resistance of *Staphylococcus aureus* to these antibiotics, have been well documented. This resistance may be associated with the production of β-lactamases and the production

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of mecA gene.[2] The burden of antibiotics resistance due to CA-MRSA is a problem in Nigeria, because antibiotics are readily available and can be purchased by any intended user.

Fluoroquinolones, are among the most commonly available classes of antimicrobial drugs in both the hospital and community pharmacy shops. Norfloxacin and Levofloxacin, with a resistant pattern of 76.9% and 65.4% each, this denotes the trend of fluoroquinolone resistance to MRSA. Waber[24] concluded that fluoroquinolones will have the dual effect of promoting *Staphylococcus aureus* while selectively eradicating Methicillin sensitive *Staphylococus aureus* (MSSA) strains but favours MRSA colonization, thus it can be postulated that the resistant patterns of MRSA observed in this study, is well associated with indiscriminate use of antibiotics in the community. The overall effect is the circulation of this infection within the populace.

CONCLUSION

Methicillin resistant *Staphylococcus aureus* has been found as a problem in lower respiratory tract infections which can be enhanced with improper therapy, long duration of treatment and lack of awareness. It is suggested that people presenting with pneumonia-like syndrome should be assessed for MRSA, and the history of their antibiotic usage fully ascertained for proper management.

Conflicts of interest: None.

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Table 1: Demographic features of participants with lower respiratory tract infections

Variables	N	MRSA isolates	P-value
Sex			
Male	80	15(9.9%)	0.001
Female	72	11(7.2%)	
Age:			
≤20	10	0	0.001
21-40	16	18(11.8%)	
41-60	23	6(3.9%)	
≥60	3	2(1.3%)	
Co-morbidity			
Diabetes	5	2(1.3%)	0.001
HBP	8	2(1.3%)	
HIV	3	2(1.3%)	
Pneumonia	20	7(4.6%)	
None	116	13(11.2%)	

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Occupation			
Students	88	10(6.6%)	0.001
Civil servants	22	3(2%)	
Famers	16	6(3.9%)	
Traders	26	7(4.6%)	

Table 2: Clinical and Social life habits and duration of illness of the subjects

Variables	Total number	MRSA isolates (%)	P-value
Duration of sickness in (wks)			
≤1	50	3(2%)	0.001
1-5	45	11(9.9%)	
≥5	17	7(4.6%)	
Unknown	40	1(0.7%)	
Previous Antibiotics used			
Prescribed by a Doctor	87	26(17.1%)	0.001
Self- medication	07	0(0%)	
None	58	0(0%)	
Length of antibiotics used in (days)			
≤5	94	5(3.3%)	0.038
6-10	16	5(3.3%)	
≥10	42	16(10.5%)	
Cigarette smoking:			
YES	01	0(0%)	0.392
NO	148	23(15.1%)	
Previously	03	3(2%)	
Alcohol Consumption			
YES	10	2(1.3%)	0.138
NO	02	2(1.3%)	
Previously	140	22(14.5%)	

Table 3: Multiple variate analysis of the risk factors for acquisition of MRSA

Variables	OR	95% C.I
Sex	13.896	1.169-1.577
Age	17.417	2.126-2.697
Occupation	8.931	1.737-2.778
Co-morbidity	15.888	3.471-4.506
Duration of sickness	15.654	1.894-2.468
Length of antibiotics used	14.964	2.047-2.699
Cigarette Smokers	44.362	2.703-2.966
Alcohol consumers	23.619	2.482-2.956

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Table 4: Antibiotic susceptibility pattern of MRSA isolates

ANTIBIOTICS	SENSITIVE (%)	RESISTANT (%)
Ciprofloxacillin	22 (84.6)	04 (15.4)
Norfloxacin	06 (23.1)	20 (76.9)
Gentamycin	17 (65.4)	09 (34.6)
Amoxicillin	02 (7.7)	24 (92.3)
Streptomycin	05 (19.3)	21 (80.7)
Rifampicin	05 (19.3)	21 (80.7)
Erythromycin	08 (30.8)	18 (69.2)
Chloramphenicol	08 (30.8)	18 (69.2)
Ampiclox	01 (3.9)	25 (96.1)
Levofloxacin	09 (34.6)	17 (65.4)

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