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Multidrug Resistance in *Staphylococcus aureus* Isolated from Patients Living and Admitted in a Hospital Located in a Gas Flaring Community

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Staphylococcus aureus* is a colonizer of the nasal cavities of humans and can cause various infections under favourable conditions. A causal relationship between ambient air pollution and antibiotic resistance is beginning to unfold.
Aim: The present study aimed to provide information on the prevalence and resistance pattern of *S. aureus* obtained from hospitalized patients living in a gas flaring location.
Methodology: Nasal swab samples were obtained from 40 patients hospitalised and living in a gas flaring environment. Microbiological isolation and identification methods were utilized to identify *Staphylococcus aureus*. Antibiotic sensitivity was assessed for each of the isolate to various commonly prescribed antibiotics s as recommended by CLSI using the disk-diffusion method.
Methicillin resistant *Staphylococcus aureus* (MRSA) was determined using cefoxitin disc and oxacillin containing agar.
Results: A total of 40 (100%) *Staphylococcus aureus* were isolated from the nasal swabs of hospitalized patients. A 72.5 %, 67.5% and 47.5% resistance of the isolates to penicillin, amoxicillin and cloxacillin was found when the samples were tested against these antibiotics. The percentage prevalence of 67.5% of *S. aureus isolate* were methicillin resistant by the cefoxitin disc method. This method was found to be more authentic than oxacillin disc methods. Multidrug resistance was observed in 75.0% of isolates.
Conclusion: This high prevalence observed may not only be associated with misuse of antibiotics but may have been influenced by gas flaring activities, as the link between pollution and increased resistance has been provided in some earlier studies. We therefore join the call to end gas flaring.

Keywords: Gas flaring; Staphylococcus aureus; multidrug resistance; resistance; hospitalized patients.

1. INTRODUCTION

Ambient air pollution is a major environmental health problem with significant role in respiratory infections, including pneumonia, chronic heart disease, obstructive pulmonary disease, asthma, stroke, and lung cancer. WHO [1] estimates about 4.2 million premature deaths occurred worldwide due to exposure to fine particulate matter. Particulate matter (PM)2·5 increases its spread after inhalation due to the presence of diverse antibiotic resistance elements. Other ambient air pollutants with respiratory system pathologies include ozone (O3), oxides of sulfur, nitrogen, and carbon. In Nigeria, the facilities for effective utilization of these gasses are lacking. Gas flaring activities carried out routinely by oil exploration companies are done close to residential quarters which poses a significant hazard to the health of populations exposed to it [2]. It pollutes the air, heats up the atmosphere and releases air pollutants.

Antibiotic resistance and air pollutants emanating from gas flared are each in their own right among the greatest threats to global health. Indiscriminate, irrational and overuse of antibiotics are well known drivers of antibiotic resistance [3,4]. In recent times there are evidence showing the contribution of PM2.5 air pollution to the spread of antibiotic-resistance. PM2.5 air pollution harbour numerous antibiotic-resistant genes [5], which when transferred between environment or inhaled cause chronic and recurrent respiratory diseases, abnormal hematological indices and other infections traceable to PM2.5 inhalations [6,7] which could bring about hospitalization.

S. aureus is unique in its capacity to thrive as a commensal organism while still maintaining its pathogenic potential and it's responsible for various forms of infection. The anterior nares being the most frequent carriage and reservoir site is a source of spread of the pathogen [8]. These bacteria can be transformed into persistent carriage state by establishing a solid interaction with nasal epithelial cells. *S. aureus* exhibits a number of properties including, antibiotic resistance, synthesis of enzymes and biofilm formation which makes them highly invasive and leads to life-threatening infections [9]. Nasal carriage is implicated as a major risk factor in the pathogenesis of *S. aureus* infections in patients with reduced immune status [10] with higher risks of infections in persistent carriers [11]. Ambient air pollutants have a direct and damaging effect on the human immune system specifically the immune organs of the respiratory tract [12].

However, the most serious problem is that *S. aureus* easily acquires resistance to many antibiotics especially from methicillin-resistant *S. aureus* (MRSA) and to drugs from other groups of antibiotics [13]. MRSA are growing threats to the public, therefore Accurate detection of MRSA in infected patients is of the utmost importance to ensure effective treatment and to prevent further transmission.

Therefore, the study was aimed at isolating *Staphylococcus aureus* from the nasal cavities of patients admitted in a hospital where gas is flared, the resistance of *Staphylococcus aureus* to methicillin and to other antibiotics.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 40 nasal swabs were collected between January to March 2022 from patient's resident and admitted in a Government General hospital located where gas flaring activities take place in Delta State Nigeria. The samples were collected using sterile swab sticks after obtaining consent from the participants who were informed about the aim and objectives of the study. The swabs were transported within 2hrs of collection.

2.2 Isolation and Identification of *Staphylococcus aureus*

The bacteriological analyses of the swab samples collected from the hospitalized patients were performed using conventional methods, as described previously [14,15]. The nasal swabs were inoculated into Brain Heart Infusion (BHI) broth (Oxoid Ltd.,UK) and incubated overnight at 37 °C. After overnight culture, the broth culture was streaked on Mannitol Salt Agar (Oxoid Ltd., UK) and incubated at 37°C for 24 to 48 h. The colonies suggestive of characteristic staphylococci morphology and presumptive isolates with yellow colonies and zones were picked and sub-cultured to obtain a pure culture. The isolated colonies were identified by conventional phenotypic methods (first by Gram staining, catalase, and coagulase reactions) as reported in standard microbiological methods [16]. Colonies were stored on nutrient agar slants until ready for further investigations.

2.3 Antimicrobial Sensitivity Testing

Antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) (Oxoid Ltd, Hampshire, UK) according to Clinical and Laboratory Standards Institute (CLSI) guideline 2016 [17]. A direct colony suspension of each *S. aureus* obtained from a sub cultured agar plate isolate was prepared matching a 0.5 McFarland standard and was plated on Mueller-Hinton agar. Multi antimicrobial disc comprising of Cloxacillin (CLX-5µg), Dicloxacillin (DCX-1µg) Gentamicin (GEN- 10 *µ*g), Cefuroxime (CEX-30µg), Erythromycin (ERY -15µg), Amoxicillin Clavulanic Acid (ACA -30µg), Streptomycin (STR- 10 *µ*g),) and Ceftriaxone (CEF-30µg) were used.

2.4 Detection of Methicillin Resistance in *Staphylococcus aureus* Using Cefoxitin Disc and Oxacillin Containing Agar

Methicillin resistance for confirmed *S. aureus* isolates was evaluated by the CLSI disk diffusion method on Mueller-Hinton agar (MHA) (oxiod, Ltd., Basingstoke, UK) using cefoxitin disc (30 µg). A 0.5 Mc Farland standard suspension of *S. aureus* was prepared and inoculated on MHA plate. The plates were incubated at 37°C for 18 to 24hr. The zone diameters were measured. An inhibition zone diameter of ≤21 mm and ≥22 mm was reported as cefoxitin resistance and susceptibility respectively.

To determine methicillin resistance using oxacillin, MHA plate prepared with 4% NaCl. were inoculated with 10 µL of 0.5 Mc Farland suspension of the *S. aureus* isolate, then an oxacillin disc (1 µg) was placed on the agar and incubated at 35°C for 24 h. After incubation, Plates were observed for growth in transmitted light as recommended by CLSI (2005) [17]. The zone size of ≥13 mm was interpreted as susceptible while ≤ 10 m was interpreted as resistant according to the CLSI recommendation. The ATCC 25923 *S. aureus* strain was used as a quality control.

2.5 Multi Drug Resistance

Multi drug resistance, defined as resistance to ≥ 3 drugs by the *S. aureus* was determine in this study.

2.6 Inclusion and Exclusion Criteria

To participate in this study, individuals must be 18 years and above and have resided

permanently close to gas flaring sites for at least two years. Visitors and individuals who were ill and bed ridden were excluded from this study.

3. RESULTS

A total of 40 nonduplicate *S. aureus* isolates were obtained from the nasal cavities of 40 patients hospitalized in a hospital located where gas flaring activities occurred. *S. aureus* identification was confirmed by colony morphology, coagulase slide test, subsequent tube test, and other biochemical tests. Results presented in Table 1 shows the demographic distribution and indicated that they were more female than male that consented to the study and *S. aureus* was isolated in all the participants, making it a 100% prevalence.

Table 2 details the results of *S. aureus* resistance to commonly used antibiotics. A higher level of resistance was found in the penicillin antibiotics. A 72.5 %, 67.5% and 47.5% resistance of the isolates to penicillin, amoxicillin and cloxacillin was found when the samples were tested against these antibiotics. Erythromycin and Cefuroxime were also among the readily available antibiotics. These antibiotics showed 40% resistance. Low resistance of 7.5% to the antibiotics streptomycin and amoxicillin clavulanic acid were observed.

Of the 40 *Staphylococcus aureus* isolated in this study, 26 (65.0%) were methicillin resistant by the oxacillin disc diffusion methods while 28 (70.0%) *S. aureus* were methicillin resistant by the cefoxitin disc diffusion method. The oxacillin disc diffusion methods incorrectly identified 2 isolates as susceptible that was cefoxitin resistant.

Multidrug resistance (MDR) (≥ 3 drugs) was detected in 75.0% (30/40) of the isolates. Resistance to 3, 4, 5 and 8 drugs was found to be 15%, 40.0%. 10% and 20.0% respectively (Table 4). In total, 14 resistant patterns were observed out of which 12 were MDR.

Table 1. Gender Distribution of *Staphylococcus aureus* from nasal cavities of admitted patients

Gender	No. of participants	No. of *S. aureus* isolated
Mail	30	30
Female	10	10
Total	40	40

Table 2. *Staphylococcus aureus* resistance to tested antibiotics

Class of antibiotics	Antibiotics	Number resistant S. aureus isolates. N = 40	Percentage
Penicillin	Penicillin (PNC)	29	72.5
Penicillin	Cloxacillin (CLX)	19	47.5
Penicillin	Dicloxacillin (DCX)	11	27.5
Penicillin	Amoxicillin (AMX)	27	67.5
Aminoglycoside	Gentamicin GEN	9	22.5
Aminoglycoside	Streptomycin (STR)	3	7.5
Beta lactam inhibitor	Amoxicillin Clavulanic Acid (ACA)	3	7.5
Cephalosporins	Ceftriaxone (CFT)	8	20
Cephalosporins	Cefuroxime (CFR)	12	40
Macrolide	Erythromycin (ERY)	12	40

Table 3. Comparison of two phenotypic methods of detection of MRSA

Method of MRSA detection	Number of MRSA detected	% prevalence
Cefoxitin disc diffusion (30 µg),	27	67.5
Oxacillin disc diffusion (1 µg)	25	62.5

Table 4. MDR profile

Staphylococcus aureus	Resistance Profile	Number of isolates	number of Antibiotics	MDR profile
1	PNC	4	1	Non MDR
2	CLX, PNC AMX	2	3	MDR
3	CLX, DCX, PNC, AMX, GEN, ACA, STR, CFT	4	8	MDR
4	PNC, AMX, CFR, ERY	8	4	MDR
5	CLX, OCX, PNC, AMX	2	4	MDR
6	CLX, OCX, PNC, AMX, ERY	2	5	MDR
7	OCX, DCX, PNC, CFR	2	4	MDR
8	OCX, AMX, GEN	2	3	MDR
9	CLX, AMX	4	2	Non MDR
10	DCX, PNC, OXA, GEN	2	4	MDR
11	CLX	2	1	Non MDR
12	CLX, DCX, PNC, AMX ERY	2	5	MDR
13	CLX, DCX, PNC, CFR	2	4	MDR
14	DC, AMX, GEN	2	3	MDR

KEYS: Cloxacillin (CLX), Dicloxacillin (DCX), Gentamicin (GEN), Cefuroxime (CFU), Erythromycin (ERY), Amoxicillin Clavulanic Acid (ACA), Streptomycin (STR), Ceftriaxone (CFT) amoxicillin (AMX, penicillin (PNC)

4. DISCUSSION

Staphylococcus aureus was the investigated bacteria in this study, and it was present in all 40-nasal swab of persons living and admitted in a hospital located in a community where gas flaring activities take place. *S. aureus* can persistently colonize the nares of humans and establish strong nasal epithelial cells interactions and also overcome host defence mechanisms. A 100% prevalence of *S. aureus* in this study may be attributable to the occurrence of a local nosocomial outbreak in the hospital that may not have been noticed and reported. *S. aureus* is one of the most common nosocomial pathogens and a normal flora of human skin. In Nigeria bacteria outbreaks are not easily noticed or reported especially if they are not epidemic prone disease outbreaks or infectious disease outbreaks. This is attributed to the non-existence or lack of the awareness of the existence of a surveillance network in hospitals. Airborne of *S. aureus* dispersion by multiple sources, such as asymptomatic health care personnels who are nasal carriers, hospital environment and supplies and equipment [18,19]. may have contributed to the high prevalence of S. *aureus among* hospitalized patients. The health status also has influence on nasal microbiota. In a study involving hospitalized and healthy individuals, the patient's microbiota was dominated by *S. aureus* and *S. epidermidis unlike the healthy individuals that were* dominated by *Actinobacteria*. Different studies have reported different prevalences of *Staphylococcus aureus* amongst hospitalized patients of gas flaring environment for example, in Central Uganda Kateete et al. [20] reported 41.9%, in Iran and Palestine, 31% was reported by Nabil et al. [21]. A high prevalence of 64% was reported in Nigeria by Akujobi et al. [22]. This shows that Nigeria has worst case scenario of *S. aureus* colonization amongst hospitalized patients living in gas flaring environment and this is a worrying situation because of its association with respiratory infections [2] and the ease of accumulating antibiotic resistance of varied antimicrobial class. In a review and meta-analysis on respiratory-related ailment found among individual's resident in states where gas is flared in Nigeria carried out by Moneke et al., [23], Delta State ranked second with a percentage prevalence of 43%. Respiratory infection has been linked with lost or weakened immune defences [24]. In addition, a weakened immune status also predisposes to other non-respiratory infection.

Colonization of patients with *S. aureus* has been linked to the occurrence of subsequent infections [25] and increasing resistance. The antibiotics to which the *S. aureus* isolated showed very high resistance included first line agents such as penicillin, ampicillin and oxacillin. Alarmingly, 72.5% of *S. aureus* were resistant to ampicillin, a

commonly used first-line antibiotics for most infections of the upper respiratory tract. These bacteria also were found to be low in resistance to its stronger alternative, amoxycillin/clavulanic acid (7.5%) which was a positive observation, making it a choice antibiotic for emperic therapy in the sampling location. A relatively low level of resistance to, amoxycillin/clavulanic acid was previously reported in the findings of Akortha and Ibadin, [26] who reported 17% in Benin, Nigeria. In contrast Onanuga and Awhowho, [27] in Akwa-Ibom, Nigeria reported very high resistance of 69.6% to amoxycillin/clavulanic acid. Whilst amoxycillin/clavulanic acid can be used as empiric treatment in the study area, it is discouraged to do so in other locations where high levels of resistance was observed. Other commonly used antibiotic such as erythromycin and cefuroxime had a 40% resistance which was high. Although, earlier report by many authors in Nigeria working in different research areas [28,29,30,31,32] have reported antibiotic abuse and high prevalence of self-medication with antibiotics as being responsible for the selection of antibiotic resistant bacterial strains. This may not be the only reason for the increase in resistance observed. The cause of this higher rate of resistance as previously reported in a similar research area of persons exposed to ambient air gas pollution is likely due to the unique exposures to flared gas pollutants, especially PM2.5 [2,24,33].

Multidrug-resistant *S. aureus* (MDRSA) is among the leading causes of morbidity and mortality in hospitalized patients, particularly due to methicillin-resistant *Staphylococcus aureus* (MRSA). In our study, MDRSA was observed in 75.0% of isolates, which is comparable to a study conducted by Wasihun et al. [34]. Of the 12 MDRSA resistance patterns, 40% and 20% of the isolates were resistant to 5 and 8 varied combinations of antibiotics respectively. This is a reflection of the high levels of inappropriate administration and over-use of antibiotics in the study area. Commercially available antibiotics are easily abused by individuals [4,29]. Moreover, the gas flared and inhaled by the inhabitants who are patients under study, may alter the genetic composition of the *S. aureus* and cause increased antibiotic resistance [2]. Causal link between natural gas flaring, increasing antibiotic resistance and human health has been provided [5].

A percentage prevalence of 67.5% of *S. aureus isolate* were methicillin resistant by the cefoxitin disc method. This method was observed to be more authentic than oxacillin disc methods. It also substantiates that mecA, which code for resistance to methicillin is a more prevalent antibiotic resistance gene detected in *S. aureus*. The 67.5% observed in this study was quite high.

Although the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) infection in Nigeria is scarce. The review by Abubakar and Sulaiman [35] evaluating the prevalence, trend and antimicrobial susceptibility of clinical MRSA isolates of twelve studies in Nigeria found that in general, the prevalence of MRSA increased from 18.3% (2009) to 42.3% (2013). These figures though shows increasing trend are lower than that obtained in our study, further highlighting the possible link of gas flaring with an increased antibiotic resistance.

5. CONCLUSION

S. aureus is a potentially harmful microorganisms that colonize the normal flora of the nares and a possible cause of respiratory and non-respiratory infection in inhabitants and hospitalized patients exposed to gas flared. The high level of colonization and antibiotic resistance observed in this study raises the alarm of an impending antibiotic resistance crisis in the region. The need for a close monitoring of inhabitants of gas flaring location not only for environmental exposures but also for antibiotic resistance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

The study was approved by the Ethical Research Committee of the Delta State University, Abraka and from the management of the hospital where the study was carried out. Participants gave Informed written consent before sample collection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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