

Original Research Article

Prevalence of Methicillin-Resistant *Staphylococcus aureus* among Healthy Residents of Ekosodin Community in Benin-City, Nigeria

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Received: 9 March 2015

Revised accepted: 28 June 2015

Abstract

Purpose: To determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in apparently healthy residents of Ekosodin community, a peri-urban settlement, in Benin City, Nigeria.

Method: Nasal swabs collected from 200 randomly selected individuals, aged between 16 and 38 years, were used in the study. Isolates from the swabs were aseptically collected and characterized using standard and established microbiological methods, which included growth and fermentation on mannitol salt agar (MSA), colonial morphology, Gram-staining reaction and biochemical tests. Antimicrobial susceptibility test was performed on Mueller-Hinton agar (MHA) by modified Kirby-Bauer disc diffusion method.

Results: *S. aureus* was isolated in 49.5 % ($n = 99$) of the 200 nasal swabs. Among these isolates, 43 % were from male residents and 22.2 % ($n = 22$) were MRSA. The MRSA isolates indicated relatively high rate of resistance to penicillins, moderate resistance to erythromycin and cefuroxime, and least resistance to gentamicin, streptomycin, ceftriaxone, ciprofloxacin and co-trimoxazole. There was no significant gender difference in terms of the colonization of *S. aureus* ($p < 0.05$).

Conclusion: Many apparently healthy residents of Ekosodin community are nasal carriers of MRSA. The need for rational chemotherapy, routine detection and regular surveillance of MRSA to limit its spread and reduce treatment failures is vital.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Nasal swabs, Multidrug resistance, Rational chemotherapy, Treatment failure, Peri-urban community.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Staphylococcus aureus is a pluripotential pathogen that is capable of causing disease utilizing a diverse range of virulence determinants. It is part of the normal nasal and skin flora in approximately 50 % of the population and can cause a wide range of infections ranging from superficial wounds, soft tissue infections, abscesses to serious infections such as otitis and infective endocarditis (IE). Methicillin-resistant

Staphylococcus aureus (MRSA) is especially resistant to a wide range of antibiotics including methicillin, oxacillin, penicillin and amoxicillin.

Acquisition of MRSA has been associated with two different environments: Community-acquired MRSA (CA-MRSA) and healthcare-acquired MRSA (HA-MRSA). MRSA infections continue to be a serious and formidable challenge to health care providers and their prevalence is increasing exponentially. Infections caused by MRSA

increase the length of hospital stays, are responsible for rising health care costs, and a high attributable mortality [1]. A large proportion of those infected with MRSA are just carriers of MRSA without being sick. Coagulase-negative staphylococci (CNS) on the other hand have long been regarded as apathogenic but their important role as pathogens and their increasing incidence have been recognized and studied in recent years.

Virulence factors of *S. aureus* include both structural components and secreted cellular products. The polysaccharide capsule facilitates resistance to phagocytosis, while enterotoxins produce sepsis syndrome by functioning as super antigens. CNS is by far the most common cause of bacteremia related to indwelling devices [2]. *Staphylococcus epidermidis* has been identified as a major cause of nosocomial infections especially in patients with predisposing factors such as indwelling or implanted foreign polymer bodies or prosthetic devices.

Data on CA-MRSA infections, usually manifested, as skin infections are increasing. There are however dearth of information on MRSA in otherwise healthy individuals in the community settings of South-South Nigeria. There is need for increase awareness and recognition of MRSA in the community which have unique properties compared with the CA-MRSA in the event of infectious outbreaks. The nasal carriage of *S. aureus* acts as a source for invasive infections and provides effective control strategies.

This study reports data on the nasal carriage of MRSA among residents of Ekosodin community, indicating some morphological and biological characteristics of public health importance.

EXPERIMENTAL

Study area

This preliminary study was conducted in Ekosodin community, a peri-urban settlement in Benin City, Nigeria. A total of 200 apparently healthy volunteers, consisting of equal number of males and females in the age range of 16 – 38 years (mean age of 23 years) were enrolled for the study with due consent. The upper anterior nares were swabbed using sterile cotton swab sticks and immediately transported to the Pharmaceutical Microbiology Laboratory of the University of Benin. The swabs were aseptically

cut into Tryptic Soy Broth (TSB) (Scharlau, Spain) and incubated at 37 °C for 24 h.

Microbiological tests

A loopful of the overnight culture in TSB was transferred onto the surface of Mannitol Salt Agar (MSA) (Conda Pronadisa, Spain) and 5 % Blood agar. These were further incubated at 37 °C for 24 h. The characteristic isolates were aseptically selected and characterized using standard established microbiological methods, which include growth and fermentation on MSA, colonial morphology, Gram staining reaction and biochemical characteristics [3]. Isolates that were Gram-positive cocci (grape-like clusters), which produce catalase and positive to both slide and tube coagulase tests with human plasma were considered as *S. aureus* in this study.

Susceptibility test

Antibiotic susceptibility test was carried out by disc diffusion method [4] using antibiotic multi-disc containing amoxicillin 30 µg, ampicillin/cloxacillin 30 µg, ceftriaxone 25 µg, cefuroxime 20 µg, ciprofloxacin 10 µg, pefloxacin 10 µg, gentamicin 10 µg, streptomycin 30 µg, erythromycin 10 µg and sulphamethoxazole/trimethoprim 30 µg.

Identification of methicillin resistance

MSA plates were prepared with ceftioxin at a concentration of 4 mg/L. The test strains were applied in the following manner: suspensions of viable organisms were prepared, matched with 0.5 McFarland standard and a loopful spread over the MSA - Ceftioxin agar plates. A control plate of blood agar was used for each strain [5]. Incubation was carried out in air (aerobically) at 35 °C for 48 h and the growth of any visible colonies after incubation was recorded as a positive. Study on resistance to methicillin (ceftioxin) was conducted on all strains of *S. aureus* and the multidrug resistant (MDR) strains among other staphylococci species isolated.

Statistical analysis

The results are presented as mean ± standard error of the mean (SEM) and N represents the total number of *S. aureus* isolates that showed resistance to methicillin. Data comparison between gender groups was carried out by Chi-square test. At 95% confidence interval, $p < 0.05$ was taken as statistically significant.

RESULTS

Of the 200 nasal swabs collected from the residents, 99 (49.5 %) yielded *S. aureus*, 47 (23.5 %) were *S. epidermidis* and 54 (27 %) were *S. lugdunensis*. Out of the 99 isolates of *S. aureus*, 22 (22.2 %) were resistant to methicillin; 1 out of 4 (25 %) of the 47 *S. epidermidis* was MDR and also showed resistance to methicillin (Table 1). Multi drug resistance in this study was defined as resistance to three or more classes of antibiotics other than the beta-lactam antibiotics.

Figure 1 shows the resistance profile of the various isolates against the antimicrobial agents tested. The resistance profile of all the MRSA strains is presented in Figure 2. MRSA isolates showed high resistance to amoxicillin (91 %), ampicillin/cloxacillin (77 %), erythromycin (86 %), cefuroxime (68 %) and least resistance to streptomycin (5 %), and ciprofloxacin (9 %). The distribution of MRSA according to age and sex is shown in Table 2. Prevalence of MRSA was higher in females than in males.

Table 1: Frequency of staphylococcal isolates showing resistance to methicillin

Isolate	Frequency (N = 200)	%	Methicillin- resistance	%
<i>S. aureus</i>	99	49.5	22	22.2
<i>S. epidermidis</i>	47	23.5	1	25.0
<i>S. lugdunensis</i>	54	27.0	0	0.0

N = total number of samples

Table 2: Distribution of MRSA among age and sex

Variable	<i>S. aureus</i> (N = 99)	%	MRSA (N = 22)	%
Gender				
Male	43	43	8	18.6
Female	56	57	13	23.2
Age (years)				
16-20	16	16	6	37.5
21-25	61	62	12	19.7
26-30	17	17	3	17.6
31-35	4	4	0	0.0
36-40	1	1	1	100

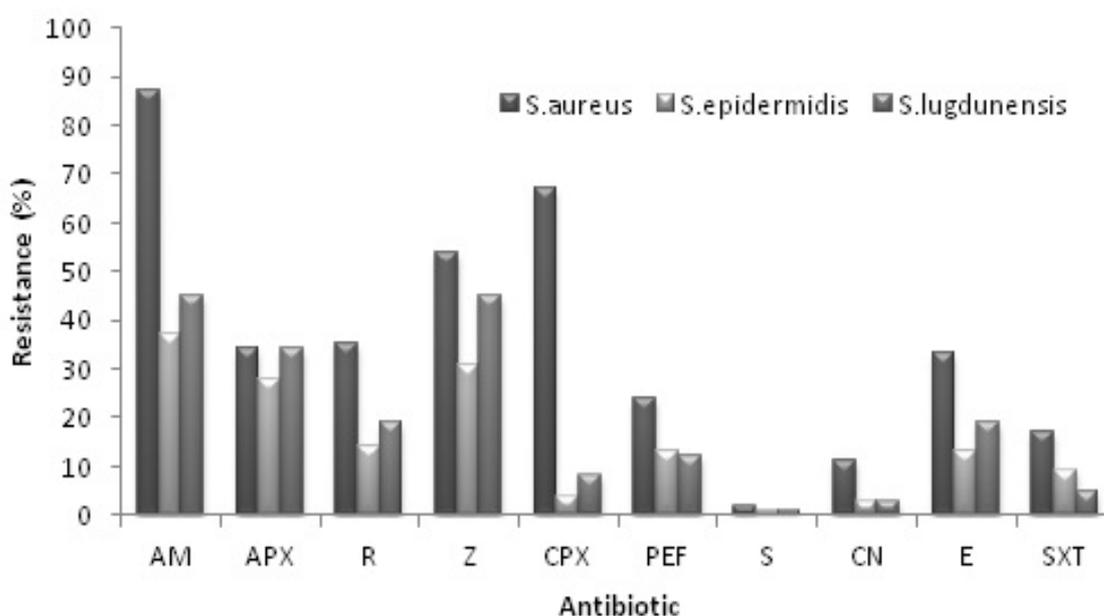


Figure 1: Resistance profile of the various isolates to the antibacterial agents used.

Key: AM-amoxicillin; APX-ampicillin/cloxacillin; R-ceftriaxone; Z-cefuroxime; CPX-ciprofloxacin; PEF-pefloxacin; S-streptomycin; CN-gentamicin; E-erythromycin; SXT-sulphamethoxazole

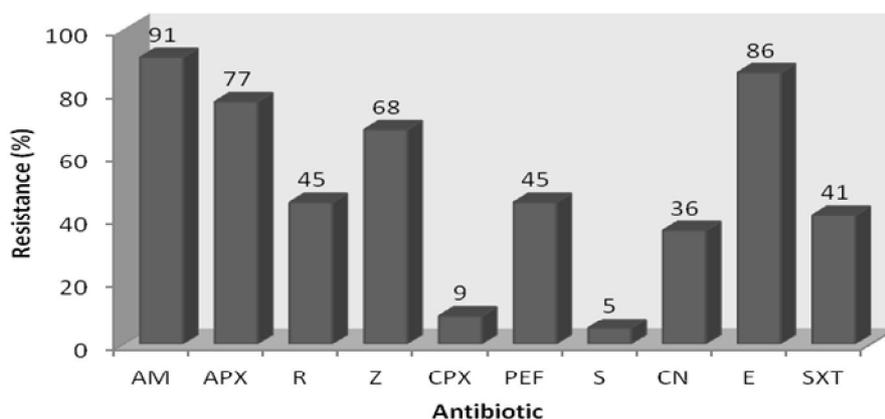


Figure 2: Antibiotic resistance profile of the MRSA isolates

Key: AM-amoxicillin; APX-ampicillin/cloxacillin; R-ceftriaxone; Z-cefuroxime; CPX-ciprofloxacin; PEF-pefloxacin; S-streptomycin; CN-gentamicin; E-erythromycin; SXT-sulphamethoxazole

DISCUSSION

The anterior nares is either one of the two external openings to the nasal cavity in the nose. The nares (nostrils) are always heavily colonized, predominantly with *Staphylococcus epidermidis* and *Corynebacteria*. This is also the main carrier site of *S. aureus* (in about 20 % of the general population). Despite intensive efforts to control resistant organisms by aggressive infections control methods antibiotic-resistant *Staphylococci* especially MRSA has become the most common cause of hospital acquired infections worldwide [6].

S. aureus is a well-known pathogen with an alarmingly increasing level of developing resistance to many available antimicrobial agents. Nasal *S. aureus* has been implicated in community associated infections like soft tissue infection and hospital infections like bacteremia [7]. The adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotic introduction [8]. The emergence of multidrug-resistant MRSA strains with unique properties has raised public health concern. Acquisition of CA-MRSA and HA-MRSA pathogens presents complications in the antibiotic management of such infections, with the attendant increase in hospital stay, cost of treatment and the morbidity and mortality of patients [9].

In this study, the prevalence of 22 % MRSA is similar to previous findings which reported 20 % MRSA prevalence rate. Chen and Huang [10] reported 13.6 % in Taiwan, but lower than that of Onanuga and Timedie [11] who reported 27.5 % in Amassoma, South-South of Nigeria. Rijal *et al* [12] had reported a much higher 56.1 % nasal microbial colonization in healthy children in

Nepal. These variations may be attributed to the characteristics of the population under study. A population that is on antibiotics as at the time of sampling may yield a much lower prevalence of *S. aureus* while a population from hospital settings may yield a much higher prevalence because of the high prevalence of infectious patients in that environment. Other factors that can cause variations may be sampling and culture techniques.

The susceptibility test results showed that the β -lactam antibiotics were the least effective agents; this has been widely reported for *S. aureus* from various sites of healthy subjects [13] and nosocomial infections. These drugs are readily available and often irrationally misused which could possibly select resistant strains [14] thereby render them ineffective in the treatment of infections caused by them.

The observed moderate resistance to erythromycin and co-trimoxazole may also be as a result of their uncontrolled usage in the environment which favors the increasing number of resistant strains due to selection pressure [10]. The low resistance observed against the fluoroquinolones, gentamicin and streptomycin support previous findings [13]. The profound activities of these drugs against the organisms may be largely due to their cost and availability in parenteral formulations requiring expert's assistance for administration. The fluoroquinolones have been known to be effective against bacteria that were resistant to other oral antibiotics but there is an increasing resistance trend to the fluoroquinolones as observed in this study. The availability of the cheaper generic formulations could possibly explain this observation. The increased level of resistance to the trimethoprim/sulphamethoxazole (TMS) and penicillins may be connected with their ready

available and affordability, since they are cheap and often purchased without physician prescription.

CONCLUSION

This study has revealed that many apparently healthy residents of Ekosodin community are nasal carriers of MSRA. The high prevalence of MRSA among the community dwellers has the potential to increase with possible resistance spreading to other infectious agents, with concomitant antibiotic treatment failures. The public health implications of this trend call for regular surveillance, enlightenment, promotion of awareness and control measures among stakeholders.

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