GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(Research Article)

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Investigation and mapping of the prevalence of "superbugs" Methicillin-resistant *Staphylococcus aureus* (MRSA) and antimicrobial susceptibility pattern in Enugu metropolis in Southeast Nigeria

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GSC Biological and Pharmaceutical Sciences, 2023, 23(03), 245–254

Publication history: Received on 06 May 2023; revised on 18 June 2023; accepted on 21 June 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.3.0239

Abstract

Global public health is threatened by increasing incidence of Antimicrobial resistance (AMR). Methicillin-Resistant *Staphylococcus aureus* (MRSA), usually referred to as "superbug, is a prominent AMR that is generating concerns across the world. They are resistant to commonly used antibiotics, thereby making treatment of common infections very difficult; resulting in prolonged illness and hospitalisation, disability and in some cases, death. Despite the concerns and threats posed by MRSA, studies of its prevalence in communities across Nigeria are scanty. Here, we investigated the prevalence of MRSA in the South East Nigeria using isolates obtained from two major hospitals in Enugu metropolis and a medical laboratory. The isolates were clinically identified from different specimens and pure isolates were subjected to antimicrobial susceptibility profiling using conventional antibiotics. Geographic Information System (GIS) techniques were used to analyse the anonymised three year MRSA records. This provided relevant insights on the spatial spread of MRSA in the city. This research serves as a first step towards modelling its spatial epidemiology in the region. Results indicated that cases of MRSA were widespread in the city. Majority of MRSA were resistant to the antibiotics indicating that they are multidrug-resistant (MDR).

Spatiotemporal analyses conducted showed that incidence of MRSA was increasing by the year.

Keywords: MRSA; Prevalence; South East Nigeria; Antimicrobial-resistance; GIS

1. Introduction

Antimicrobial resistance (AMR) is used to describe health situations when microorganisms mutate as they come in contact with antimicrobial drugs (e.g antivirals, antibiotics, antifungals, antimalarials, and antihelmintics) thus, rendering medicines ineffective in treating infections. Such antimicrobial resistant microorganisms are commonly referred to as superbugs [1]. Antimicrobial resistant microorganisms are usually found in people, animals, food, and can spread from person to person likewise between people and animals. Although AMR occurs naturally over time, usually via genetic mutations, the increasing rate has been attributed to the misuse and overuse of antimicrobials especially, when it is given without professional advice and when they are used to promote growth in animals and fishes [2, 3].

AMR has drawn global concerns due to the fact that new resistance mechanisms are emerging and spreading globally, threatening the ability to treat increasing range of infectious diseases. This inability often results to prolonged illness and hospital stay, extra cost of treatment, disability, as well as death [1]. This puts the gains of the Millennium

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Development Goals (MDG) at risk, while endangering achievement of the Sustainable Development Goals (SDG). In addition, AMR is threatening the success of critical medical procedures such as organ transplantation, cancer chemotherapy, diabetes management and major surgeries, because without effective drugs for prevention and treatment of infections, such procedures become compromised.

One of the prominent AMR organisms that has been categorised as posing serious threat is Methicillin-Resistant *Staphylococcus aureus* (MRSA). It is commonly found on the skin and in the noses of healthy people. It causes a range of infections from mild skin infections and food poisoning to life threatening infections [4, 5] such as surgical wound and bloodstream as well as pneumonia. It is still a significant organism with persistently increased morbidity and mortality rate worldwide, despite the advances in infection prevention, modern medicine, active surveillance efforts and the ongoing development of new antibiotics.

MRSA is widespread across the globe, and its prevalence has increased in health-care, community and livestock. The prevalence varies geographically, more in Europe, North and south America, but has been detected sporadically in Asia and Africa [6-9].

The understanding of the prevalence of MRSA at regional levels is very important because of regional variations in the prevalence and spread that is dependent on various environmental and risk factors. In Nigeria for instance, MRSA is among the most frequently encountered bacterial species in microbiology laboratories that causes significant epidemiologic and therapeutic problems in the country [10, 11]. Studies of MRSA prevalence in Nigeria have been conducted mostly in the South Western (Ibadan, Ilorin) and Northern part of the country [12-14]. However, comprehensive data on the prevalence and molecular epidemiology of this pathogen in south-eastern Nigeria especially, Enugu state is very limited. Therefore, this research aimed on ascertaining the prevalence of MRSA in clinical specimens in Enugu, southeast Nigeria. This research is part of a broader study aimed at obtaining a comprehensive understanding of the spread of the pathogen in the region, their resistance or susceptibility to prominent antibiotics and development of alternative therapy to combat it.

2. Materials and methods

2.1. Study area

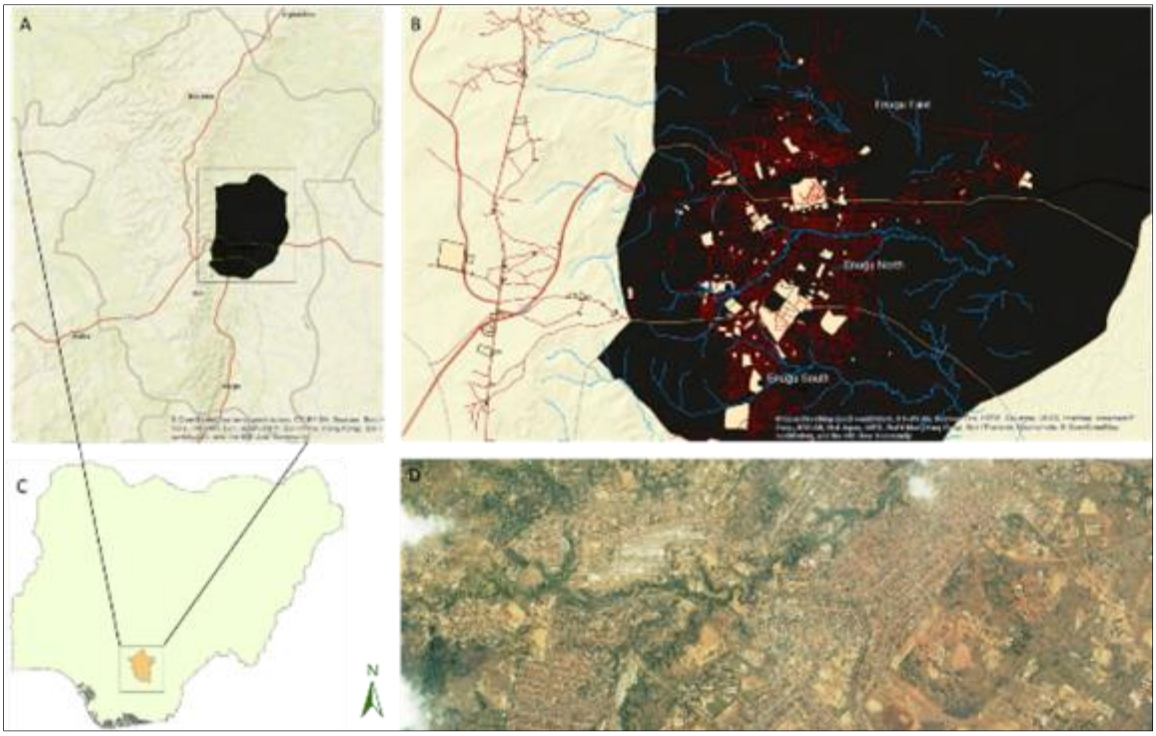

Figure 1 Maps showing various aspects of the study area. A: shows the map of Enugu State, B: Enugu metropolis, C. Map of Nigeria with Enugu State inset, D. High resolution satellite image of Enugu metropolis obtained from QuickBird imagery

Enugu metropolis, which is the biggest urban area in the region, was used as a case study area, representative of prevalence in other parts of the region. It is a key city in south Eastern Nigeria, due to political, academic and natural resource reasons. It is currently the capital of Enugu State (Figure 1). Enugu Metropolis is made up of 3 out of the 17 LGAs in the state namely: Enugu North, Enugu East and Enugu South. It has a population of over 700,000 based on the last census conducted in Nigeria 2006, and an area of 556Km^2 [15].

The metropolis rose to geopolitical prominence due to the discovery of coal in commercial quantities in 1909. This brought about the emergence of a permanent cosmopolitan settlement, making it the oldest urban area in the Igbo-speaking southern part of the country [16]. It was therefore natural that it became the administrative capital of the Eastern Region, the Republic of Biafra, and subsequently the East Central State, Anambra State and currently Enugu State. The state is within the tropical rain forest belt with mostly tropical savannah climate. The climate is characterised by the rainy and dry seasons, with mean temperature ranging between 30.64 °C and 15.86 °C, with a very high humid that peaks between March and November.

Due to the prominence of Enugu metropolis, it has continued to draw a lot of people from the rural areas and other parts of the region, leading to its urbanisation. Recent increase in commercial, academic, and industrial activities has facilitated the urbanisation process. However, this has exacerbated environmental problems in the metropolis and exerted a lot of pressure on local resources.

2.2. Data

The main data used in this study were clinical isolates obtained from two tertiary hospitals and a molecular pathology laboratory in the city. A total of 1,294 isolates of *S. aureus* previously isolated from different clinical samples (collected from April, 2012 to December, 2014) at University of Nigeria Teaching Hospital (UNTH), Annunciation Specialist Hospital, and the Safety Molecular Pathology Laboratory Services Ltd were used for the study. The clinical samples were: urine, seminal fluid, skin scrapings, catheter tips, sputum and, then swab samples from vagina, urethra, endocervix, ears, throat, wounds and eyes. Additional data such as political map of the state and street map of the metropolis were used for the GIS analysis. The agar media used include: Mueller Hinton Agar (MHA), Mannitol salt agar (MSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) Agar Base (Acumedia, Michigan, USA), while the broth media used were: Brain Heart Infusion (BHI), Lactose broth, Sucrose broth and Glucose broth. All the media were prepared according to the manufacturer's instructions. Cloxacillin sodium (Nichben Pharm. Co Nig Ltd) was obtained and used as pure drug. Antimicrobial sensitivity discs containing cefixime (5µg), Co-trimoxazole (30µg), clindamycin (10µg), gentamycin (10µg), ciprofloxacin (5µg), cloxacillin (30µg), ofloxacin (5µg), ceftriaxone (30µg), norfloxacin (10µg), and erythromycin (10µg) (Oxoid, UK) were also used to determine the susceptibility profile.

2.3. Methods

The isolates were inoculated onto MRSA agar plates, each containing cloxacillin sodium 600 µg/ml in 1 litre agar and incubated for 24 h at 37 °C [17]. Representative colonies were subjected to preliminary tests, such as Gram staining, catalase, coagulase, glucose, lactose, sucrose and mannitol fermentation tests for species identification. The colonial growths were stored in aliquots containing 85% glycerol mixed with 15% distilled water at 4 °C. Prior to use, an aliquot of the test isolates were sub-cultured onto fresh MRSA agar containing 600 µg/ml of cloxacillin sodium in 1 litre agar and then incubated for 24 h at 37 °C for reactivation. Reactivated cultures were standardized by growing the organism aerobically at 37 °C in a shaker water bath for 16 ±2 h to a cell density of 2.0 x 10^{8}cfu/ml [18].

2.3.1. Susceptibility testing of the S. aureus isolates to antibiotics

The antimicrobial susceptibility profile of the isolates to the antibiotics was determined using the Kirby-Bauer disc diffusion technique (described as follows). 0.1 ml of standardized MRSA cultures were diluted with sterile saline to get the turbidity match of 0.5 McFarland standards and dispensed unto dried agar plates. These were dispersed evenly onto the agar surface using sterile swab stick to make a bacterial lawn. Inoculated plates were allowed to dry for 15 mins and the antibiotic discs were aseptically placed on the inoculated plates at 15 mm away from the edge of the plates. The plates were allowed a further drying period of 30 mins and then incubated for 24 h at 37 °C. After incubation, zone of clearance/inhibition was observed and the diameter of the zone was measured, recorded and compared with a standard for each drug. The isolates were recorded as resistant, intermediate or susceptible based on the standard interpretative chart as described by the Clinical Laboratory Standard Institute [19]. Using these methods, samples were classified into Methicillin-Sensitive *Staphylococcus aureus* (MSSA) and Methicillin-Resistant *Staphylococcus aureus* (MRSA).

2.3.2. Prevalence and incidence of MRSA and MSSA

The frequency of occurrence of MRSA and MSSA isolates obtained from these samples was ascertained for each year of study. Age and sex distribution of patients records from where the samples were obtained were also analysed. Demographic characteristics were statistically analysed with Chi-square and Fisher's exact test (two tailed) using SPSS statistical program. All reported *P* values are two-sided and a *P*-value of less than or equal to 0.05 ($P \leq 0.05$) is considered to be statistically significant.

2.3.3. Mapping of MRSA prevalence

The MRSA prevalence data was further analysed and sorted into years and street addresses. The street addresses of samples identified to be MRSA were geocoded using GIS techniques. In order to enhance anonymity, geocoded coordinates of the incidents of occurrence of MRSA were mapped to the nearest neighbourhood zones. Maps showing the annual prevalence of MRSA at the zonal levels were subsequently produced. Other maps showing prevalence based on age, gender, occupation and type of sample were also produced, to provide a broader understanding of the spatial distribution of the pathogen.

3. Results

The spatial variation of the isolates based on different clinical samples (Table 1) from where MRSA were isolated is shown in Figure 2. The highest number of MRSA isolates (70.65 %) was obtained from urine samples followed by high vaginal swab (19.06 %).

Table 1 Frequency of MRSA isolated from 1,294 different clinical samples

Clinical samples	Number	% number
Urine	730	70.65
High vaginal swab	197	19.06
Wound swab	22	2.09
Urethral swab	34	3.34
Ear swab	3	0.31
Endo cervical swab	3	0.31
Semen	22	2.14
Sputum	14	1.31
Skin scrapping	2	0.16
Throat swab	2	0.21
Catheter tip	2	0.21
Eye swab	2	0.21
Total	1033	100 %

The sensitivity result of the organisms to various drugs also indicated that 83% of MRSA were resistant to the antibiotics (cefixime, Co-trimoxazole, clindamycin, gentamycin, ciprofloxacin, cloxacillin, ofloxacin, ceftriaxone, norfloxacin, and erythromycin) and none was fully susceptible to all the tested antibiotics. Invariably, all the MRSA could be taken as multidrug-resistant (MDR) from these results.

The prevalence of MRSA and MSSA isolates are shown in Table 2. Figure 3 also shows the spatiotemporal variations of the pathogens. A greater number of the sampled *S. aureus* were MRSA. The prevalence of MRSA (89%) 2014 is higher than that of 2013 (84%) and 2012 (72%). This was confirmed to be statistically significant by a Chi Square test that revealed a p-value of 0.0001. The spatial distribution of the pathogen indicates that MRSA incidents are widespread, with incidents recorded in various parts of the study area. However, more incidents of MRSA were recorded around Emene followed by Nike parts of the metropolis.

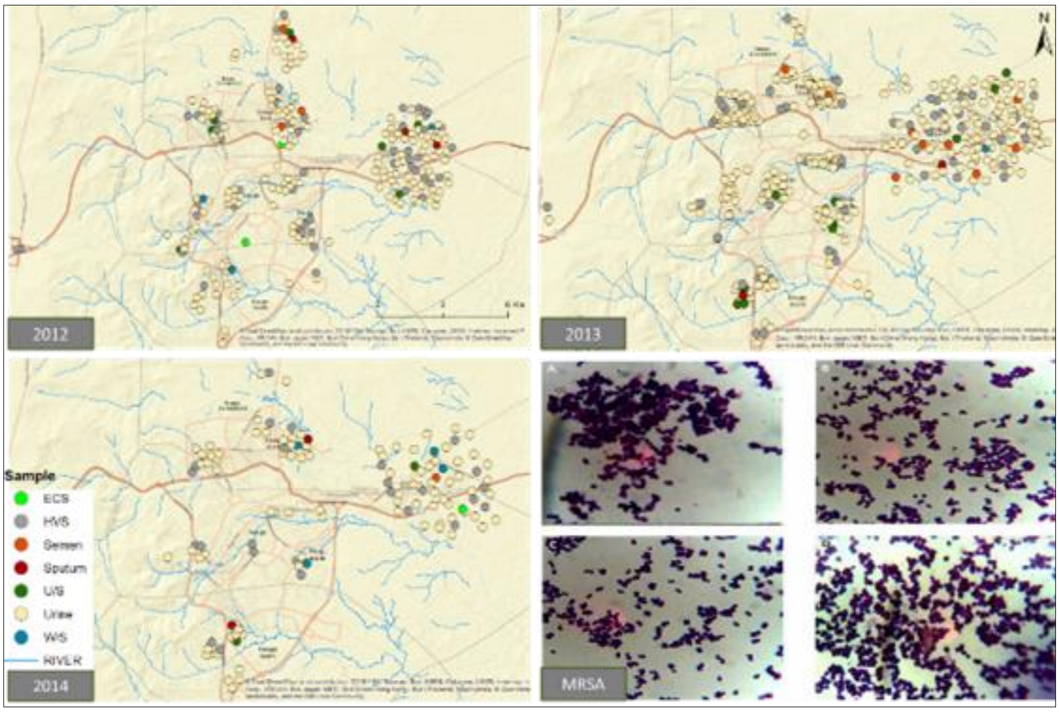

Figure 2 Spatiotemporal distribution of MRSA prevalence by clinical samples used for MRSA isolation. Inset is gram stained pictures of MRSA isolated from different clinical samples (A - Urine, B - High Vaginal Swab (HVS), C – Wound Swab (W/S), D – Semen). Note: ECS= Endo Cervical Swab, and U/S = Urethra Swab

Table 2 The prevalence of MRSA among the *S. aureus* isolates

Year	Total no of *S. aureus*	MSSA	% MSSA	MRSA	%MRSA
2012	485	138	28	347	72
2013	629	103	16	526	84
2014	180	20	11	160	89

Key: MSSA = Methicillin sensitive *S. aureus*, MRSA = Methicilin resistant *S. aureus*

Table 3 shows the demographics of the prevalence based on the different age groups and gender of patients. Approximately 5% of MRSA isolated came from patients between the age groups of 0-10 and 11-20, 34% of the isolates came from the 21-30 age group, 31% from the 31-40 age group, 10% age group 41-50, and 15% from age groups of 51 years and above. The highest susceptible age group of patients to MRSA is 21-30 years followed by 31-40 years, while the least were in the age groups of 0-20 years. The spatial distribution of prevalence by age is shown in Figure 4.

Table 3 Age and sex distribution of patients with MRSA

Age (years)	Male	% male	Female	% female	Total number	% total Number
≤ 10	16	1.55	33	3.19	48	4.64
11-20	16	1.55	40	3.87	56	5.42
21-30	37	3.58	311	30.11	350	33.88
31-40	80	7.75	240	23.23	321	31.07
41-50	35	3.39	74	7.16	107	10.35
≥ 51	67	6.49	84	8.13	151	14.63
Total	251	24.31	782	75.69	1033	100

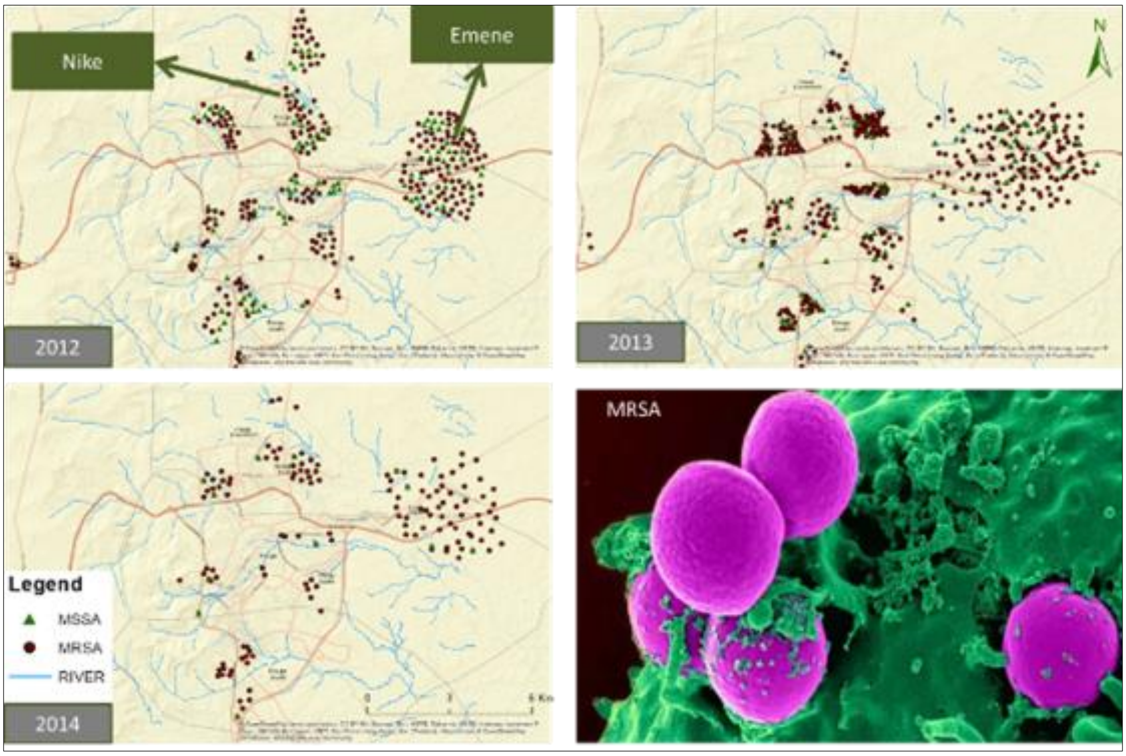

Figure 3 Spatiotemporal distribution of the prevalence of sampled MRSA and MSSA in Enugu metropolis

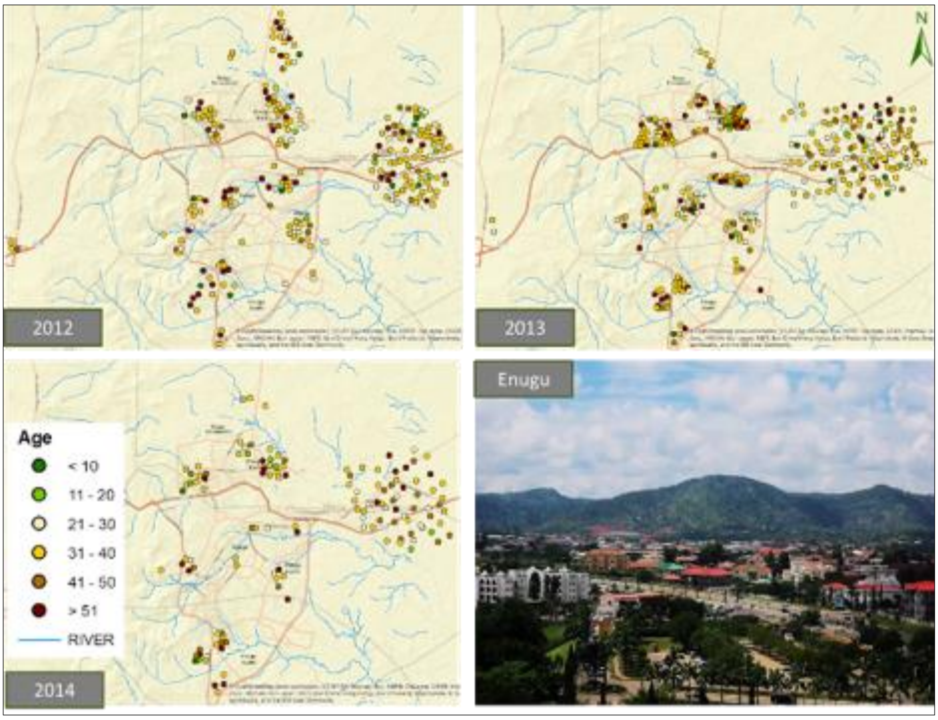

Figure 4 Spatiotemporal distribution of MRSA prevalence by age. Inset is a section of Enugu metropolis

Furthermore, more MRSA isolates (75.69%) were isolated from female patients than male patients (24.31%). This gender variation was replicated across all the age groups and neighbourhoods (Figure 5). However, the highest prevalence of MRSA in females was found in the age group of 21–30 years (30.11%) while the highest in males was between the age group of 31-40 years (7.75%).

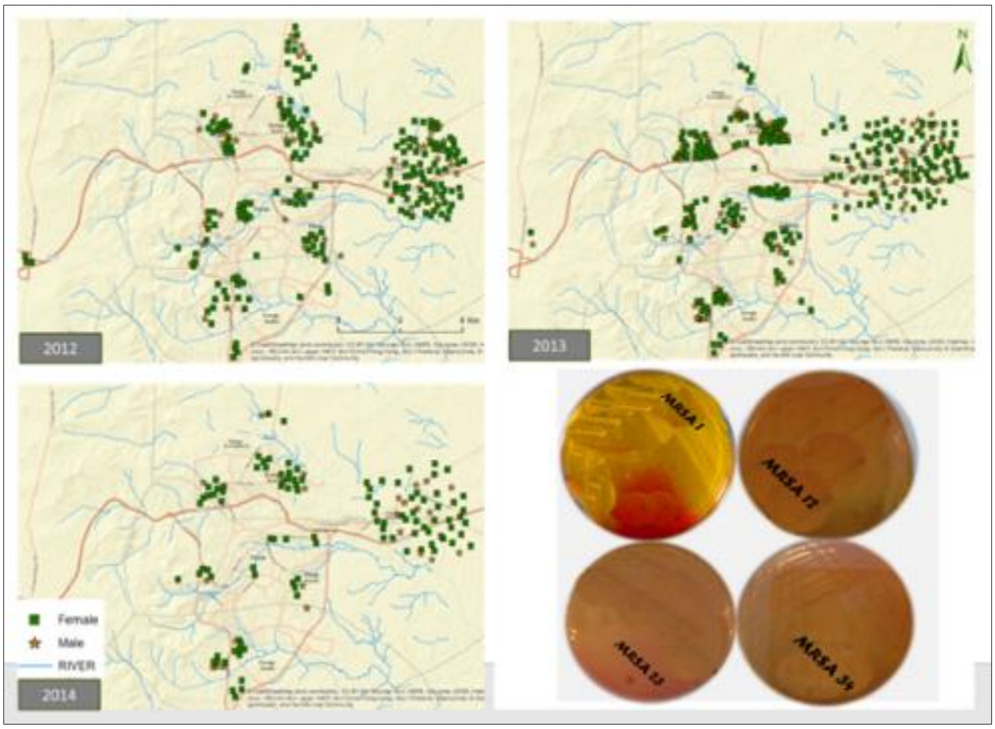

Figure 5 Spatiotemporal distribution of MRSA prevalence by gender. Inset is Pictures showing mannitol fermentation of some isolated MRSA

In terms of prevalence across different occupations, majority of the isolated specimens came from public/civil servants, business people, and students (Figure 6).

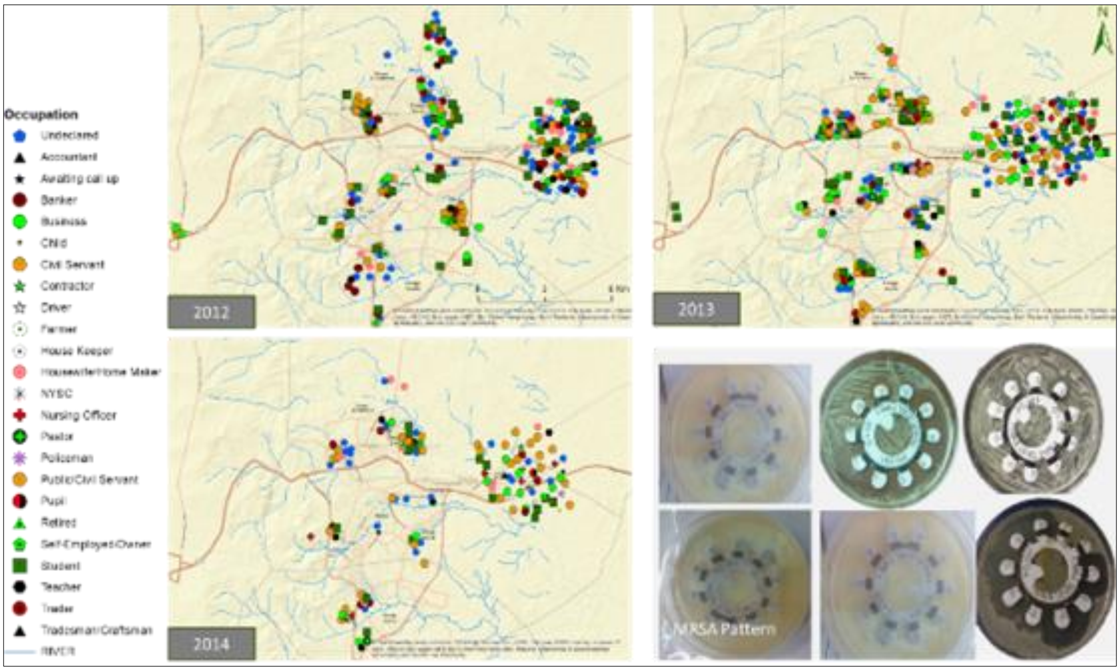

Figure 6 Spatiotemporal distribution of MRSA prevalence by occupation. Inset is antibiotic sensitivity plates showing resistance pattern of MRSA to various antibiotics

4. Discussion

The isolation of MRSA from different clinical samples (Table 1) demonstrates that MRSA may colonize different parts of the body, such as the genitourinary tract, hands, axillae, perineum, wounds, catheter sites, throat and gastrointestinal tract as has been found by some other researchers [20, 21]. The antibiotic sensitivity study observed a relationship between methicillin resistance and resistance to other antibiotics. Others have similar result as well [22, 23]. Results obtained from this research indicate that MRSA occurrence is very common and widely distributed across the neighbourhoods within the locality and its spread is increasing by the year. The observation of more incidents of MRSA around the Emene and Nike zones might be due to the proximity to one of the hospitals from where the samples were obtained. The results obtained from the study area reflect a probable distribution of MRSA across the state. This widespread nature of the pathogen is alarming and required concerted efforts to curtail its spread.

The female patient predominance observed in this study correlates with other findings which revealed that the frequency of urinary tract infection (UTI) of which *S. aureus* is one of the predominant organisms isolated, is greater in females as compared to males [24, 25]. This is due to anatomic and physical factors [26]. Other factors could be pregnancy and sexual activity [27, 28]. On the other hand, the highest prevalence of MRSA in females was found in the age group of 21–30 years (30.13%) and 31-40 years (7.78%) in males; this is probably due to the fact that sexual activity is higher within those age limits. This agrees with other reports [26, 27, 29].

Despite the fact that MRSA is not a new clinical disease, its incidence continues to grow at an alarming rate and the prevalence rate shows high regional variance [30 – 32].

In Enugu (South-East), results obtained from this study show that the prevalence of MRSA isolates are reasonably high. The high prevalence observed in this study and other studies indicate that MRSA continues to be a worldwide menace. This is particularly important for Nigerian health facilities where hygienic procedures are not strictly adhered to. Hence, health providers and the government should enforce strict adherence to relevant policies in order to minimize the spread. The prevalence across different occupations is a reflection of the demographic distribution in the state which has more of civil servants, students and business people.

5. Conclusion

This research investigated the prevalence of MRSA in south east Nigeria by deploying a combination of relevant clinical and geospatial techniques. While clinical techniques provided definitive methods for the isolation and specie identification of the pathogen, GIS was deployed to spatially analyse and map the confirmed incidents of occurrence of MRSA. This research found that MRSA is widespread in the study area with high prevalence that is increasing by the year. The rate of prevalence was higher in female patients than male patients. This was found in the age group of 21–30 years and 31-40 in males indicating that the spread might be as a result of unprotected sexual activities. The findings of this research indicate the need for coordinated action against the emergence and spread of this globally recognised organism.

Antimicrobial resistance (AMR) organism is a complex problem that affects all facets of the society and driven by many interconnected factors. Single, isolated interventions have limited impact. Countries need to develop national action plans as well as strengthen health and surveillance systems to combat AMR threat, as currently being championed by the World Health Organisation (WHO; 2018). In addition more concerted efforts towards greater innovation and investment are also required in research and development of new antimicrobial medicines, vaccines, and diagnostic tools. Furthermore, the use of antibiotics in both humans and animals should be strictly regulated to prevent abuse.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the staff of the departments in all the hospitals and the laboratory where the research was conducted.

Disclosure of conflict of interest

There is no competing interest in the publication of this paper.

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