GET Journal of Biosecurity and One Health

GET Global Emerging Pathogens Treatment Consortium

GET JOURNAL OF BIOSECURITY & ONE HEALTH

PUBLISHED BY:

Global Emerging Pathogens Treatment Consortium

JOURNAL WEBSITE

www.getjournal.org

Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* from Urine of Patients Attending Ajikobi Hospital, Ilorin, North Central, Nigeria

Omotosho AO[1,2*] and Bale MI[1,3]

[1]Department of Microbiology, Faculty of Pure and applied Sciences, Kwara State University, Malete, P.M.B 1530, Ilorin, Kwara State.

[2]Department of Environmental Risk Management, School of Social and Environmental Sustainability, University of Glasgow, DG1 4ZL, Dumfries, Glasgow.

[3]Department of Microbiology and Parasitology, College of Medicine and Health Sciences, University of Rwanda.

Corresponding Author: Omotosho Abass Olawale

ORCID: 0000-0003-3139-3647

ABSTRACT

Antibiotic resistance is becoming the next public health emergency as opportunistic pathogens such as *Staphylococcus aureus* are gaining resistance to frontline antibiotics. This study aimed to determine the prevalence and multidrug-resistant *Staphylococcus aureus* in urine samples of patients attending Ajikobi Cottage Hospital in Ilorin, Kwara State. A total of 170 urine samples from male and female patients of 10-70 years age groups were aseptically collected and cultured on mannitol salt agar for isolation. Biochemical tests were carried out for identification, and antbiotic susceptibility patterns of the isolates were determined using Kirby-Bauer disc diffusion technique. A total of 46 (27%) *S. aureus* were obtained, 40 (36%) from females and 6 (10%) from males. The highest occurrence was recorded between the ages of 21-30 and 31-40 years, with 35% and 23%, with females in these groups accounting for 55% and 22%, respectively. This accounts for a high-rate of bacterial infection amongst sexually active women of child bearing age groups. This was followed by 19% and 17% for age groups 10-20 and 41-50, respectively. The lowest incidence from this research was recorded in age groups above 50 years, with 0%. Antibiotics sensitivity profiles of the 46 isolates of *S. aureus* tested showed that 71% were resistant to ampicillin, followed by 50% resistance to erythromycin, 43% to amoxicillin, followed by 26%, 19%, 13% and 8.6% resistance to cefoxitin, gentamicin, ciprofloxacin and vancomycin respectively. A total of 5 (10.8%) multidrug-resistant *S. aureus* was recorded from this study. Resistance to vancomycin from this study is of public health concern that requires due attention, as vancomycin is a last-resort antibiotic used to treat serious infections.

Keywords: Antibiotic Resistance; Bacteriuria; Erythromycin; *Staphylococcus aureus*; MRSA; Urinary Tract Infections; Vancomycin.

Corresponding Author's Email: Olawaleabass08@gmail.com

GET Journal of Biosecurity and One Health (2023), 2(2), 1-8.
DOI:10.36108/GJOBOH/3202.20.0210

INTRODUCTION

Globally, about 150 million people are diagnosed with urinary tract infections (UTI) each year, costing the global economy over 6 billion U.S. dollars [1]. The emergence of antibiotic resistance is continually increasing this estimate as opportunistic pathogens such as *Staphylococcus aureus* is gaining resistance to frontline antibiotics, causing treatment difficulty [2]. Bacterial infections of the urinary tract in humans are the most frequent bacterial disease affecting outpatients, hospitalized patients, and apparently healthy populations; and they are more common in females than males as a result of the shortened urethra [3].

Staphylococcus aureus, a Gram-positive facultative anaerobic bacterium, is a relatively uncommon cause of urinary tract infection in the general population [4]. However, isolation of *S. aureus* from urine samples, which is often secondary to staphylococcal infection, arises from major risk factors such as diabetes, sickle cell disease, anatomical malfunction of the urinary tract, poor toilet habits, pregnancy in women, and prostate enlargement in men [5].

Urinary tract instrumentation and indwelling catheter increase the risk of *S. aureus* carriage in the urinary tract [6]. Some studies have shown the relationship between bacteriuria and bacteremia, and urinary tract infection by *S. aureus* have been identified as a clinical entity and could result from urinary tract colonization with *S. aureus* [7].

Urinary tract infections are often treated with broad-spectrum antibiotics, even in situations where narrow spectrums can be appropriate, because of concerns about infections with resistant organisms [7]. The development of resistance to many antibiotics by *S. aureus* has involved the acquisition of determinants by horizontal transfer of mobile genetic elements [8]. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) causes different types of infections that are very difficult to treat in humans because this bacterium has developed the mechanism to resist the action of antimicrobial agents such as penicillin, methicillin and cephalosporin and other antibiotics in use, especially that of the beta-lactam class [9, 10].

MRSA is responsible for a wide range of infections, including bacteremia, endocarditis, osteomyelitis, meningitis, septicemia, pneumonia, and bacteriuria [11]. Strains of *S. aureus* resistant to multiple antimicrobial agents constitute a significant threat to the public health care [12]. Hence, the need to address *S. aureus* multi-drug resistance. The present study determined the prevalence and antibiotic sensitivity pattern of *S. aureus* from urine samples of both male and female patients at Ajikobi Hospital in Ilorin, Kwara State to various frontline antibiotics and also determined multidrug-resistant isolates from these samples.

METHODOLOGY

Place of Study: The research was carried out at Ajikobi Cottage Hospital, Ilorin and Microbiology Laboratory, Department of Microbiology, Kwara State University, Malete, Kwara State, Nigeria. This study was carried out in four months (October 2019 to March 2020). Ajikobi Cottage Hospital is a public hospital in Omoda Area, Okekere, Ilorin West Local Government, Kwara State. It was established on January 1st, 1984, and operates on a 24-hour basis.

Collection of Samples

We visited Ajikobi Cottage hospital Ilorin, Kwara State, for sample collection. The ethical approval was received from the Kwara State Ministry of Health (**Ethical Approval Code: ERC/MOH/2019/10/116)**. With a 170 total study population, urine samples were collected from male and female wards at the hospital, with 60 (35%) male distribution, and 110 (65%) female distribution. All patients admitted to the hospital and those receiving antimicrobial treatment in the three months preceding sample collection were excluded from the study. Additionally, patients who did not show a cooperative attitude or refused to provide necessary information were also excluded. All consented patients, including outpatients and admitted patients were included in the study urine samples were collected aseptically into sterile bottles using standardized sampling techniques. The samples obtained from different patients were labelled and tagged with appropriate information.

Preparation of the Media

The workbench was disinfected using cotton wool soaked in 70% ethanol. The media (Mannitol salt agar, Muller Hilton agar and Nutrient agar) were prepared according to the manufacturer's specification by weighing a known gram of the agar medium and dissolving in the accurate medium of diluents, then agitated to mix and homogenized using a magnetic stirrer hot plate. The media were later sterilized by autoclaving at 121^{o}C for 15 minutes. The sterilized agar was then suspended at 44^{o}C in a water bath to obtain molten agar before being used [13].

Preparation of the Samples

The urine samples collected were centrifuged to wash and remove unwanted supernatants such as protein, nitrite, blood etc., in the urine samples. The pellet was mixed with 100 ml of normal saline, and then the stock saline solution was diluted.

GET Journal of Biosecurity and One Health (2023), 2(2), 1-8.
DOI:10.36108/GJOBOH/3202.20.0210

Serial dilution was carried out, and 9 ml of sterile distilled water was pipetted into five sterile test tubes. One milliliter of the stock sample in saline solution was added into the first tube using a sterile pipette and was agitated. One milliliter was pipetted from the first test tube into the second test tube, and the procedure was repeated till the 5th test tube was reached using a sterile pipette. This process was carried out on all samples.

Isolation and Maintenance of *Staphylococcus aureus*

Exactly 0.1ml (loopful) of the mixture of each of the dilutions in the tubes was transferred from dilution 10^{-3} and 10^{-5} from each sample and streaked onto well-labelled sterile pre-set mannitol salt agar plates. The plates were then inverted and incubated at 37ºC for 24-48 hours. After the incubation period, the plates were removed from the incubator, and the colonies were observed and recorded. Distinct colonies that were light yellow to whitish cream and 1-2 mm in diameter on each plate were thereafter aseptically picked and streaked onto mannitol salt agar plate to obtain pure isolates. After that, the plates were incubated for 24 hours at 37ºC. The appearance of the colonies on mannitol salt agar, their relative size, colour, texture, opacity, surface elevation, edge and shape were observed and then used to clarify their own growth pattern and to identify the bacterial types [13]. Slants were obtained by dispensing 20 ml of molten nutrient agar into adequately washed and sterilized McCartney bottles and allowed to set in a slanting position. The distinct pure isolates were aseptically inoculated into the bottles and then incubated at 37ºC for 24 hours. The bottles containing isolates were then kept in the refrigerator for further use.

Identification of Isolates

The presumptive isolates of *Staphylococcus* spp. were identified as *Staphylococcus aureus* based on morphology, catalase, and coagulase test as recommended by Bergey's Manual of Systematic Bacteriology [14].

Antibiotic Sensitivity Test

Antibiotics susceptibility test was conducted on all *S. aureus* isolates (n = 46) obtained from the study. The isolates were tested against seven antibiotics discs using the Kirby-Bauer disk diffusion method [14]. The following antibiotics disks (Oxoid disks) with their corresponding concentrations were used; vancomycin (30 µg), erythromycin (15 µg), gentamycin (10 µg), ampicillin (10 µg), amoxicillin (30 µg), ciprofloxacin (30 µg) and cefoxitin (30 µg). Mueller-Hinton agar was sterilized for 15 minutes at 121ºC, allowed to cool to about 50ºC. An 18–24-hour old pure isolate was inoculated onto Mueller-Hinton agar plate. The bacterial cultures on the plates were swabbed with a sterile swab on Muller Hilton plates. Plates were left at room temperature to remove excess moisture. With sterile forceps, different antibiotics were placed on respective bacteria plates and kept in the refrigerator for 30 minutes for pre-diffusion of the disc. Incubation was carried out for 24 hours at 37ºC. Following incubation, the inhibition zone was reported as the diameter of the zone surrounding the individual disk in which bacterial growth was absent. Based on this, the isolates were defined as resistant and susceptible according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for Gram-positive bacteria [13].

RESULTS

In this study, a total of 46 *Staphylococcus aureus* were isolated from 170 urine samples based on morphological characteristics on mannitol salt agar (MSA). Six (6) isolates were obtained from 60 male urine samples, and 40 isolates were obtained from 110 female samples. Based on physiological, morphological and biochemical tests isolates, the colonies were Gram-positive, non-motile, cocci (Grape-like clusters), catalase and coagulase positive. The colonies were large (2-4mm), circular, convex, smooth, shiny, opaque, and easily emulsifiable. Most strains produced golden yellow pigment on MSA. After the application of other identification tests, including the catalase and coagulase test, all the 46 isolates were confirmed as *S. aureus.* Table 1, 2 and 3 show the gender distribution of patients with *S. aureus* bacteriuria, age distribution of patients with *S. aureus* bacteriuria, and age distribution of patients with *S. aureus* bacteriuria in relation to gender respectively.

Gender Distribution of *Staphylococcus aureus*

The gender distribution of *Staphylococcus aureus* isolated from 170 urine samples is shown in Table 1. The results show that a total of 46 (27%) *S. aureus* were obtained, 40 (36%) from females and 6 (10%) from males.

Age Distribution of Patients with *Staphylococcus aureus*

Table 2 shows the age distribution of patients with *Staphylococcus aureus* bacteriuria. The highest occurrence was recorded between the ages of 21-30 and 31-40 years, with 35% and 23%, This was followed by 19% and 17% for age groups 10-20 and 41-50, respectively. The lowest incidence from this research was recorded in age groups above 50 years, with 0%.

GET Journal of Biosecurity and One Health (2023), 2(2), 1-8.
DOI:10.36108/GJOBOH/3202.20.0210

Age Distribution of Patients with *Staphylococcus aureus* bacteriuria in Relation to Gender

The highest age distribution of patients with S. *aureus* in relation to gender from this research was recorded in the 21- 30 age group, with female accounting for 55% to 8% male as shown in Table 3 . This was followed by age groups 41-50 and 10 – 20, with females accounting for 25% to 0% male, and 24% females to 7.6% respectively. However, there was high age distribution recorded in males in 31-40 age group with males accounting for 25% to 22% females. This was attributed to the number of males (8) to females (27%) within this age group. The lowest age to gender distribution was recorded in age groups above 50 years, with 0%.

Antibiotics Susceptibility *Profiles of Isolates*

The sensitivity patterns of *Staphylococcus aureus* isolated from 170 urine samples to different antibiotics are shown in Table 4. These data revealed that from the 46 isolates of *S. aureus* tested, 71% are resistant to ampiclox, followed by 50% resistance to erythromycin, 43% to amoxicillin, 26%, 19%, 13%, and 8.6% are resistant to cefoxitin, gentamycin, ciprofloxacin and vancomycin respectively. A total of 5% multidrug-resistant was recorded. Figure 1 shows graphical representation of the test results.

DISCUSSION

Staphylococcus aureus is a bacterial infectious agent causing bacteriuria with high prevalence in various communities and healthcare institutions; urinary tract infection caused by *S. aureus* in humans and animals is gradually becoming more challenging to treat due to the emergence of antibiotic resistance [2].

The results obtained in this study showed the prevalence of *S. aureus* from urine samples of male and female patients at Ajikobi Hospital in Ilorin, Kwara State, the sensitivity pattern of *S. aureus* to various antibiotics and multidrug-resistant isolates from these samples. Total of 46 (27%) *S. aureus* were isolated from 170 urine samples of both males and females with 10-70 age groups. This is lower than the trend 39% reported in Ghana in 2017 [15]. The higher incident occurrence from their study showed a high-level misuse of antibiotics in the region and could also be as a result of the sample type analyzed (swabs). Also, a study carried out by Sakoulas and Moellering [16] showed that the prevalence of S*. aureus* was 33.5%, the increase in percentage from their study is attributed to the population size (333) of the study. However, two independent studies showed that there was a minute prevalence 0.45% (44/3149) and 0.5-6% of *S. aureus* in urine samples of patients with risk factors (such as catheterization) for urinary tract colonization [17, 18].

From our findings, *S. aureus* was recovered more in females with 40 (36%) than males with 6 (10%) (Table 1). These findings agree with a 2013 report [5], with females having 41% to the males having 8%. However, this report disagrees with a 2019 study which showed that out of 27 patients examined, 63% of 18(27) males were diagnosed with community-acquired S. *aureus* bacteriuria, most of which were of old age (Median:61) with significant risk factor such as catheterization [19]

Furthermore, our study showed highest occurrence between the ages of 21-30 and 31-40 years, with 35% and 23%, with females in these groups accounting for 55% and 22%, respectively. The higher occurrence in females from our study could be attributed to the proximity between the genital tracts, urethra and anus, which perhaps facilitate auto transmission, as earlier suggested by [19]. The moist environment of the female perineum could also favour microbial growth and bladder contamination [20]. This study also supports a report that there is a high bacterial infection rate amongst sexually active women of childbearing age [5].

This was followed by 19% and 17% for ages 10-20 and 41-50, respectively. The lowest incidence from this research was recorded in age groups above 50 years, with 0% as shown in table 2 and 3.

Our study further revealed that, from the 46 isolates of *S. aureus* tested, 71% are resistant to ampiclox, followed by 50% resistance to erythromycin, 43% to amoxicillin, followed by 26%, 19%, 13% and 8.6% resistance to cefoxitin, gentamycin, ciprofloxacin and vancomycin respectively (Figure 1). This agrees with a meta-analysis of various studies (45 inclusions) in Ethiopia carried out by [21]. Their analysis reported that various studies showed very high resistance to Ampiclox (77%), ampicillin (75%), erythromycin (41%), relatively low resistance was observed with ciprofloxacin (19%), and the resistance level of vancomycin was (11%). Another study showed high resistance, with 79.5% to erythromycin and 100% to amoxicillin [22]. An antimicrobial study in the State of Parana Brazil, showed lower resistance to erythromycin (9.86%) and gentamycin (2.86%) [23]. This is similar to the findings of a study on the susceptibility pattern which showed that only 0.7% of the S. *aureus* strains were resistant to gentamycin [24], but contrast markedly to American study, that showed highest resistance to erythromycin. The resistance to two antibiotics (erythromycin and gentamycin) in our study may be due to indiscriminate use of antibiotics over the years.

GET Journal of Biosecurity and One Health (2023), 2(2), 1-8.
DOI:10.36108/GJOBOH/3202.20.0210

Finally, a total of 5 (10.8%) multi-drug resistant isolates were recorded from this study (Table 4).

The presence of *Staphylococcus aureus* in the urine of both male and female significantly causes urinary tract infections. Opportunistic pathogen could be enhanced by certain risk factors such as pregnancy in women, anatomical malformation of the urinary tract, immunocompromised patients suffering from diabetes and sickle cell disease, and prostate enlargement in men [24]. Drug resistant strains could cause treatment difficulty which could eventually lead to significant cases including bacteraemia [23]. From this study and various studies across the globe, it is evident that S. *aureus* is relatively susceptible to vancomycin, ciprofloxacin and cefoxitin. However, *S. aureus* is highly resistance to ampicillin, amoxicillin, and erythromycin. The current slight resistance to vancomycin from this study as well as different studies [21, 22, 23] evaluated, is bothersome, and requires due attention.

CONCLUSION

In conclusion, the threats posed by staphylococcal infections calls for adequate preventative and control measures to reduce transmission and infection. These findings will be useful to identify the challenges of the development of the drug resistance in bacteria with special references to *S. aureus.* More notably, the judicious use of antibiotics coupled with the elimination of sub-standard pharmaceuticals from drug market is pivotal to the control of antibiotics resistance in our environments.

RECOMMENDATIONS

The spread of resistant organisms should be prevented through enlightenment on antibiotic use, stoppage of over-the-counter sales and proper hygiene practices. This study strictly recommends that urine culture and sensitivity should be done when UTI is suspected to guide clinicians and physicians in taking treatment decisions. Additionally, there should be continuous monitoring of bacterial susceptibility to antibiotics before treatment prescription, to ensure adequate treatment of UTI and reducing antibiotic resistance spread.

More importantly, females should wipe from front to back to avoid spreading bacteria from rectal area to urethra, after urinations to prevent UTI. Both genders should always keep the genital area clean and dry. Females are advised to always change their tampons and pads regularly during their periods and also avoid prolonged exposure to moisture in the genital areas. Research-wise, more studies should be carried out on Staphylococcal infections, to provide new therapeutic strategies in treating resistant staphylococcal infections.

Table 1. Gender distribution of patients with *Staphylococcus aureus* bacteriuria

Sex	Examined	Isolated %
Male	60	6 (10%)
Female	110	40 (36%)
Total	170	46 (27%)

GET Journal of Biosecurity and One Health (2023), 2(2), 1-8.
DOI:10.36108/GJOBOH/3202.20.0210

Table 2. Age distribution of patients with *Staphylococcus aureus* bacteriuria

Age	Examined	Isolated (%)
10 – 20	42	8(19%)
21 – 30	83	29(33%)
31 – 40	35	8(22%)
41 – 50	6	1(16%)
≥ 50	4	0(0%)

Table3. Age distribution of patients with *Staphylococcus aureus* bacteriuria in relation to gender.

Age	Examined Male	Examined Female	Isolate Male%	Isolate Female%
10 –20	13	29	1(7.6%)	7(24%)
21 –30	36	47	3(8%)	26(55%)
31 –40	8	27	2(25%)	6(22%)
41 –50	2	4	0(0%)	1(25%)
≥ 50	1	3	0(0%)	0(0%)
Total	60	110	6	40

Table 4. Antibiotics sensitivity of *Staphylococcus aureus* isolates from male and female urine samples

Class of Antibiotics	Antibiotics	(n)	/N (%)
B-Lactams	Ampicillin	32	32/46 (71%)
Macrolides	Erythromycin	23	23/46 (50%)
B-Lactams	Amoxicillin	20	20/46 (43%)
B-Lactams	Cefoxitin	12	12/46 (26%)
Aminoglycosides	Gentamicin	8	8/46 (19%)
Quinolones	Ciprofloxacin	6	6/46 (13%)
Glycopeptides	Vancomycin	4	4/46 (8.6%)

5 isolates were found resistant to all antibiotics tested; 5/46 (10.86%) multidrug resistant isolates.

Key: n = number of resistant isolates N= total number of isolates %= percentage of resistance

GET Journal of Biosecurity and One Health (2023), 2(2), 1-8.
DOI:10.36108/GJOBOH/3202.20.0210

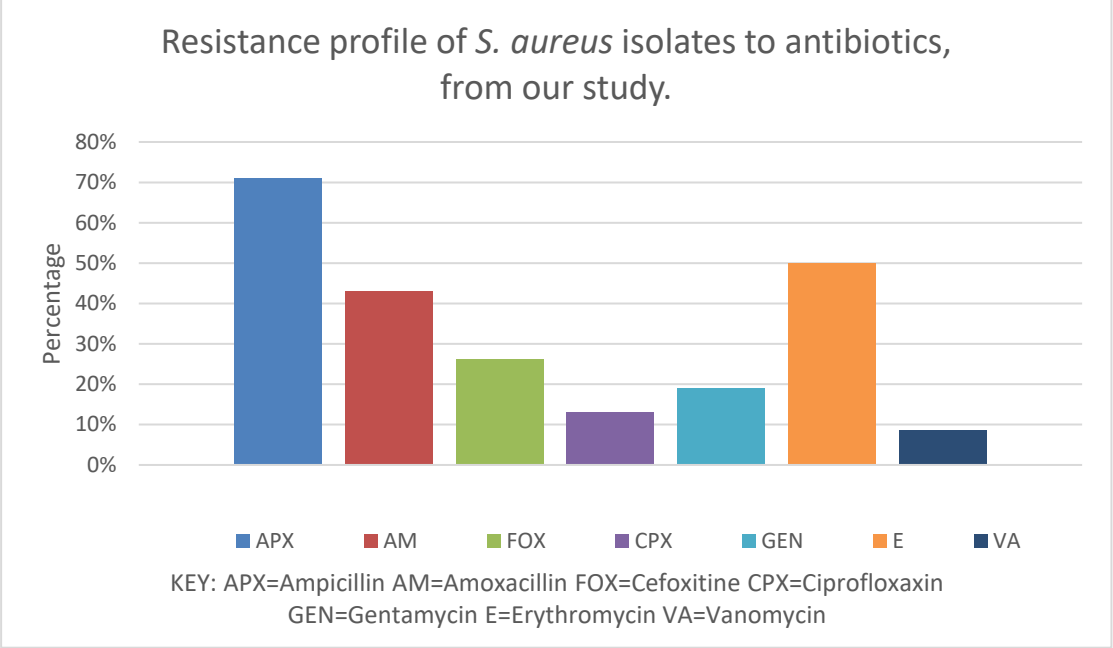

Figure 1: Resistance profile of *S. aureus* isolates to antibiotics

ABBREVIATIONS

MRSA: Methicillin Resistant *Staphylococcus aureus*

MSA: Mannitol Salt Agar

NCCL: National Committee for Clinical Laboratory Standards

UTI: Urinary tract infections

WHO: World Health Organization

AUTHORS' CONTRIBUTION

BMI participated in experimental design, overseeing execution of experimentation, collation of data, ethical approval and sample collection; OAO participated in sample collection, experimentation, collation of data and manuscript preparation. Both authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We acknowledge the members of staff of Ajikobi hospital, Department of Microbiology, Kwara State University, and the Kwara State Ministry of Health for their contributions towards the success of this research work.

FUNDING

This research received no funding.

DECLARATION OF CONFLICT OF INTEREST

The authors report no conflict of interest in this work.

ETHICAL APPROVAL

The ethical approval was received from the Kwara State Ministry of Health - **Ethical Approval Code: ERC/MOH/2019/10/116.**

REFERENCES

[1] Warren JW, Damron D, Tenney JH, Hoopes JM, Deforge B, Muncie HL. Fever, Bacteremia, and Death as Complications of Bacteriuria in Women With Long-Term Urethral Catheters. J Infect Dis. 1987; 155:1151–1158.

[2] Gould SWJ, Cuschieri P, Rollason J, Hilton AC, Easmon S, Fielder MD. The Need for Continued Monitoring of Antibiotic Resistance Patterns in Clinical Isolates of *Staphylococcus aureus* from London and Malta. Ann Clin Microbiol Antimicrob.2010; 9:10 doi:10.1186/1476-0711-9-20

[3] Sheffield JS, Cunningham FG. Urinary Tract Infection in Women. Obstet Gynecol J. 2005; 106:1085-1092.

https://doi.org/10.1097/01.AOG.0000185257.52328.a2

[4] Chambers HF. The Changing Epidemiology of *Staphylococcus aureus*. Emerg Infect Dis. 2001; 7:178- 182.

[5] World Health Organization. Antimicrobial resistance. 2011. www. *who.int/drug resistance*.

[6] Amengualue OE, Crabtree BF, O'Connor PJ, Klenzak S. Clinical Risk Factors for Methicillin-Resistant *Staphylococcus aureus* Bacteriuria in a Skilled-Care Nursing Home. Arch Fam Med**.** 2013; 3:357–360.

[7] Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, et al. Suspected Transmission of Methicillin-Resistant *Staphylococcus aureus* Between Domestic Pets and Humans in Veterinary Clinics and in the Household. Vet Microbiol. 2006; 115:148–155

[8] Aklilu E, Zunita Z, Hassan L, Chen HC. Phenotypic and Genotypic Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Dogs and Cats at University Veterinary Hospital, Universiti Putra Malaysia. Trop Biomed. 2010; 27(3): 483-492

[9] Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R. Changes in the Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Intensive Care Units in US Hospitals. Clin Infect Dis**.** 2006;42: 389-491.

[10] Sakoulas G, Moellering RC. Increasing Antibiotic Resistance Among Methicillin-Resistant *Staphylococcus aureus* Strains. Clin Infect Dis. 2008; 46: 360-367.

[11] Gould SWJ, Cuschieri P, Rollason J, Hilton AC, Easmon S, Fielder MD. The Need for Continued Monitoring of Antibiotic Resistance Patterns in Clinical Isolates of *Staphylococcus aureus* from London and Malta. Ann Clin Microbiol Antimicrob*.* 2010; 9:9-20.

[12] Bergstrom R. The Role of the Pharmaceutical Industry in Meeting the Public Health Threat of Antibacterial Resistance. Drug Resist Updat. 2011; 14: 77-78.

[13] Olutiola SL, Payne DN. Isolation Techniques: Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization. Blackwell Publishing Ltd. 2012;8-97.

[14] Cheesbrough M. District Laboratory Practice in Tropical Countries, Cambridge United Press, U.K. 2000; 27, pp.105**.**

[15] Courage K, Setsoafia S, Jean KA, and Stephen WK. Prevalence and Pattern of Antibiotic Resistance of *Staphylococcus aureus* Isolated from Door handles and Other Points of Contact in Public Hospitals in Ghana. Antimicrob Resist Infect Control. 2017; 6: 44. doi: 10.1186/s13756-017-0203-2

[16] Sakoulas G, Moellering RC. Increasing Antibiotic Resistance Among Methicillin-Resistant *Staphylococcus aureus* Strains. Clin Infect Dis. 2008; 46: 360-367.

[17] Gorgani N, Ahlbrand S, Patterson A, Pourmand N. Detection of Point Mutations Associated with Antibiotic Resistance in *Pseudomonas aeruginosa*. Int J Antimicrob Agents. 2009; 34: 414-418.

[18] Hoekstra KA, Paulton RJL. Clinical Prevalence and Antimicrobial Susceptibility of *Staphylococcus aureus* and *Staphylococcus intermedius* in Dogs. J Appl Microbiol. 2002; 93:406–413.

[19] Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Community-Associated Methicillin-Resistant *Staphylococcus aureus* Isolates Causing Healthcare-Associated Infections. Emerg Infect Dis**.** 2007;13: 236-242.

[20] Audu BM, and Kudi AA. Microbial Isolates and Antibiogram from Endocervical Swabs of Patients with Pelvic Inflammatory Disease. J Obstet Gynaecol. 2004**;** 24: 161-164.

[21] Davis SL, Perri MB, Donabedian SM, Manierski C, Singh A. Epidemiology and Outcomes of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infection. J Clin Microbiol**.** 2007; 45: 1705–1711**.**

[22] Sarawit D, Fekadu S, Astatkie A. Resistance of *Staphylococcus aureus* Antimicrobial Agents in Ethiopia: A Meta-Analysis. Antimicrob Resist Infect Control. 2017; 6:85. doi: 10.1186/s13756-017-0243-7.

[23] Eshetie S, Tarekegn F, Moges F, Amsalu A, Birhan W, Huruy K. Methicillin Resistant *Staphylococcus aureus* in Ethiopia. BMC Infect Dis. 2016; 16(1):689. doi: 10.1186/s12879-016-2014-0.

[24] Christiana A, Odeta Perpetual, Obasi O. Burden of Urinary Tract Infection (UTI) Among Female Students: South Eastern Nigeria Side of the Story. Int J Trop Dis Health. 2016; 33:2. Doi:10.9734/jjtdh/2016/22390

[25] Zavadinack NM, Herreiro F, Bandeira COP, Ito Y, Ciorlin E, Saqueti EE*. Staphylococcus aureus*: Antimicrobial Resistance Pattern. J Clin Microbiol. 2001; 23:709–712.

[26] Diamantis ML, Ortega-Loayza AG, Morrell DS. Update on the Characterization of *Staphylococcus aureus* Skin Infections in a Pediatric Dermatology Tertiary Health Care Outpatient Facility: Antibiotic Susceptibility Patterns and Decreased Methicillin Resistance. J Am Acad Dermatol. 2011; 64:440–441.