

Research Paper

Antibiotic resistance patterns and plasmid profile of methicillin resistant *Staphylococcus aureus* isolates from human samples

ABSTRACT

Antibiograms and plasmid profile are commonly used to characterize MRSA epidemiologic studies. However, antibiograms are frequently inadequate to accomplish the differentiation. Plasmid profile being more informative has been reported to be useful in tracing the epidemiology of antibiotic. Antibiotic resistance patterns and plasmid profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from human samples were investigated. Specimens were analyzed using disc diffusion assay and restriction enzymes analysis of plasmid DNA procedure. The 51 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were grouped into 18 groups using their resistogram. Twenty four (47.1%) strains out the 51 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates harbored plasmids. Single plasmid isolates were 14(27.5%), double plasmid isolates were 6(11.8%) while tripple plasmid isolates were 4(7.8%). The 24 isolates containing plasmids were categorized into 14 groups based on their resistogram. Plasmid profile showed greater similarity between isolates (10 profiles) than antibiotic resistance pattern which showed a higher disparity (14 patterns). However, resistance to various antimicrobial agents was not consistent with the presence of plasmids. No particular molecular size plasmid could be associated with any particular antimicrobial resistance patterns. Resistance was observed in isolates with various molecular size plasmids as well as in those that had no plasmids. Nonetheless, 2 pairs of isolates with the same plasmid profile also have similar (almost the same) resistance pattern. Plasmid profile analysis in conjunction with the antibiotic resistance typing is valuable in the epidemiological investigation of methicillin-resistant *Staphylococcus aureus* (MRSA).

Keywords: MRSA, antibiogram, plasmid profiling, human isolates.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*), a spherical aerobic Gram-positive, catalase positive oxidase positive, non-motile, non-spore forming coccus [1,12,26], is an opportunistic pathogen in human [1,9] and animal [12,24]. The pathogen is responsible for broad spectrum of human and animal diseases ranging from skin infections [9] to such severe disease as pneumonia [11,25], endocarditis [10], osteomyelitis [12,20], septicaemia and enterocolitis [3]. Such that infections involving antibiotic resistance strain may impact on

human health [21]. Methicillin-resistant *S. aureus* (MRSA) is responsible for hospital-acquired (HA) infections [13, 16] and presently community acquired (CA) infections [15,17,18,29]. Hospital associated (nosocomial) staphylococcal infections have been reported to be resistant to as many as 20 antimicrobial compounds, including antiseptics and disinfectants. Resistance to penicillin among *S. aureus* strains appeared a few years after the introduction of penicillin therapy. Introduction of other antibiotics such as streptomycin, tetracycline and chloramphenicol, and the macrolides was similarly followed by emergence of resistant organisms. Resistant organisms that had acquired resistance to these antibiotics were reported to be usually resistant to penicillin through the production of penicillinase. This resulted in the evolution of organisms with a wide spectrum of resistance and a marked ability to survive and spread in the hospital environment. Such multiply resistant *S. aureus* strains were of global significance as early as 1950s [19].

The genetic exchange of plasmids containing antibiotics resistant determinants between bacteria is believed to play a critical role in the evolution of antibiotic resistant bacteria and this has been shown in *S. aureus* [2]. Plasmids may contain resistance genes for single or multiple antimicrobial agents and they have been reported to transfer these resistance genes from one bacteria to another. Indeed, resistance within the staphylococci to several therapeutically useful antibiotics, including streptomycin, rifampicin, fusidic acid and novobiocin is thought to be derived from chromosomal mutation.

Molecular techniques such as plasmid profiling [4, 10], restriction endonuclease analysis of plasmid DNA [2,27], and analysis of chromosomal DNA after enzyme restriction [23,28] are currently being used in differentiating strains of bacteria isolates and opinions vary as to the discriminating power of the various types of these techniques [2], while others have opined that no single technique was clearly superior to the other for typing *S. aureus* and that a combination of different techniques is sometimes necessary [2]. Plasmid profiles have been useful in the epidemiological surveillance of disease outbreaks and in tracing antibiotic resistance [6].

Individual plasmid mediated resistance in MRSA isolates [6] and plasmid carried by resistance isolates [10,29], have been studied. At present, MRSA has become an endemic pathogen worldwide [22] and has also become multidrug resistant [14]. This study was therefore conducted to analyze the antibiotic resistance and resistance plasmids of MRSA isolates from healthy humans and consequently determine the discriminatory power of plasmid profile analysis in conjunction with antibiotic susceptibility pattern (antibiogram) in differentiating different strains of methicillin-resistant *S. aureus* (MRSA).

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 84 pathological specimens (nose and ear swab, urine) were obtained from otherwise healthy individuals in Uturu community, Abia State Nigeria with the use of sterile swab sticks and universal bottles. The specimens were labeled accordingly with the first initial of the site of specimen collection (N for nose, E for ear and U for urine). Each specimen was streaked on mannitol salt agar (MSA), nutrient agar (NA) and incubated at 37°C for 24 hours at the Department of Plant Science and Biotechnology Laboratory, Abia State University, Nigeria.

2.2 Bacterial Identification

Colonies that appeared white or yellow or cream-coloured on MSA plates with yellow surrounding (indicative of fermentation) were considered as *S. aureus* and stock cultures were immediately prepared. Isolates were further identified as *S. aureus* by testing their ability for DNase production on agar plates. Gram stain was performed and isolates were

identified by their ability to produce catalase, coagulase and oxidase enzymes then identification was finally confirmed by Bergey's manual [5].

2.3 Oxacillin Susceptibility Testing

The oxacillin disc used was procured from Oxoid, Germany. The antimicrobial susceptibility profile of the isolates was determined using the disc diffusion technique assay as described by Orji *et al.* [20]. All discs used were prepared by MAYO diagnostic laboratory, Nigeria.

With a sterile wire loop, few colonies of each of the isolates were emulsified in 5ml of sterile peptone water to a turbidity corresponding to 0.5 McFarland standard (corresponding to approximately 10^8 cfu/ml). Then 0.5 ml of each inoculum was dispensed unto the surface of dried Mueller Hinton agar plate using sterile Pasteur pipette. These were spread evenly on the agar surface with sterile swab stick (one for each inoculum). The excess inocula were discarded into a disinfectant jar. The inoculated plates were kept on the bench for 3 minutes to dry. The oxacillin disc (1 µg) was then placed centrally on the inoculated plates aseptically using a sterile forceps.

The preparations were incubated aerobically for 24 hours at 35°C. The diameter of zone of inhibition produced by each of the disc was measured, recorded and the isolates were classified as resistant (≤ 10 mm) or sensitive (≥ 13 mm) based on the standard interpretative chart as described by the Clinical and Laboratory Standard Institute [7,8].

2.4 Susceptibility Testing of MRSA Isolates to other Conventional Antibiotics

The following antimicrobial sensitivity discs were used; ampicillin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), erythromycin (15µg), gentamicin (10µg), vancomycin (30µg), amoxicillin (10µg), and ceftriaxone (30µg), to determine the susceptibility profile of all the methicillin-resistant strains using the disc diffusion technique assay as described by Orji *et al.* [20].

With a sterile wire loop, few colonies of each of the isolates were emulsified in 5ml of sterile peptone water to a turbidity corresponding to 0.5 McFarland standard. Then 0.5 ml of each of the inoculum was dispensed unto the surface of dried Mueller Hinton agar plates using sterile Pasteur pipettes. These were then spread evenly on the agar surface with sterile swab sticks. The excess inocula were discarded into a disinfectant jar. The plates were kept on the bench for 3 minutes to dry. The various discs were placed on the inoculated plates at 25 mm away from one another and 15 mm away from the edge of the plates aseptically using a sterile forceps.

The preparations were incubated aerobically for 24 hours at 35°C. The diameter of the zone of inhibition produced by each of the discs was measured, and recorded and interpreted based on the standard interpretative chart as described by the Clinical Laboratory Standard Institute [7].

2.5 Plasmid Profile Analysis

Plasmid DNA was isolated as described by Birnboim and Doly modified by use of lysostaphin for lysing the cell wall as described by Ombui *et al.* [19]. The analysis was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan. Each MRSA strain was inoculated into 3 ml tryptic soy broth and incubated overnight on a roller drum at 37°C. About 1.5 ml of each overnight broth culture was transferred into eppendorf tubes and centrifuged for one minute at 15000 rpm at room temperature. The supernatant was discarded and 2 µl of lysotaphin solution (1.0 µg/ml in distilled water) added to the pellet. Tubes were capped, vortexed and placed in ice for 30 minutes. Two hundred microlitres of alkaline detergent solution (0.2 N NaOH; 1% SDS) was added and tubes inverted several times and then kept in the water bath for five minutes. One hundred and fifty microlitres of 3 M Sodium acetate (pH 4.8) was added and tubes inverted several times to mix and then kept

in ice for at least 10 minutes. The tubes were centrifuged at room temperature at 15000 rpm for five minutes and the supernatant transferred into new eppendorf tubes. One millilitre of 95% ice cold ethanol was added to the tubes, which were then kept at -20°C for five minutes. After five minutes, they were centrifuged at 15000 rpm for three minutes, supernatant discarded and the sediment re-suspended in 40 µl of sterile distilled water and 10 µl of Tris/ETDA (10 mM Tris HCl and 1 mM ETDA. pH 8.0) was added as well. This plasmid DNA was subjected to electrophoresis according to the method mentioned below for molecular weight estimation or be stored at -20°C.

2.6 Restriction Endonuclease Analysis of Plasmid DNA

One hundred µl of Tris/ETDA (10 mM Tris HCl and 1 mM ETDA. pH 8.0) buffer were added to about 100 µl of the plasmid DNA solution that remained after the molecular weight determination, and this solution was mixed. One millilitre of 95% ice cold ethanol was added to the tube, which was then kept at -20°C for five minutes. After five minutes, it was centrifuged at 15000 rpm for three minutes, supernatant discarded. The pellet was dried by evaporation under a vacuum and dissolved in 40 µl of autoclaved water. For restriction enzyme analysis, 10 µl of 5 x –concentrated restriction endonuclease assay buffer and 5 U of endonuclease solution (EcoRI) was added to the material and allowed to react for 3 hours. The assay buffer used and the reaction temperature were according to the instructions of the manufacturer. After digestion, the sample was subjected to electrophoresis as described below.

2.7 Agarose Gel Electrophoresis

Electrophoresis was carried out in a horizontal agarose gel electrophoretic unit. The agarose gel (0.8%) was prepared in Tris-Boric acid-EDTA (TBE) buffer, allowed to cool and 3 drops of ethidium bromide was added. Using a micropipette, 20 µl of each sample plasmid preparation, with added 0.2 µl bromophenol blue dye (tracking dye) was applied into the horizontal well containing 50 ml of agarose gel. Into the first well was added 20 µl of DNA molecular Weight Marker (1kb DNA ladder) to which 0.2 µl of bromophenol blue had also been added as the standard. Electrophoresis was performed at 120v at room temperature for 2 hours. The electrophoretic bands were visualized with the aid of UV light transilluminator and the photograph was taken using a digital camera.

2.8 Molecular Weight Estimation

The size of each plasmid was estimated by directly comparing with the molecular weight marker bands (ladder 1kb) which has 13 distinctive observed bands with molecular weight ranging from 300bp-1kb. Each strain of MRSA was characterized by the number of EcoRI and size of plasmid DNA fragment it contained. Isolates with the same number and size of EcoRI plasmid DNA fragment (same plasmid profile) were considered the same strain.

3.0 RESULT AND DISCUSSION

Pathological specimens from 84 healthy individuals were examined and amongst them, 69(82.1%) were positive for *S. aureus* and 51(60.7%) were methicillin (oxacilin)-resistant. All the 51 MRSA isolates were grouped into 18 groups following their antibiotic resistant patterns. Eighteen isolates were resistant to ampicillin and amoxicillin while only 9 isolates were resistant to ampicillin alone (table 1). Resistance to ampicillin cut across all groups.

183 Table 1: The resistogram of MRSA isolated from healthy individuals
184 n = 51

Isolates	Resistance pattern
18	Ap, Am
9	Ap
3	Ap, Va, Am
2	Ap, Am, Ce
1	Ap, Ge
4	Ap, Va
2	Ap, Ci, Of, Er, Ge, Va, Am, Ce
1	Ap, Ci, Er, Ge, Va, Am, Ce
1	Ap, Of, Er, Va, Am, Ce
1	Ap, Ci, Er, Va, Am, Ce
1	Ap, Er, Ge, Va, Am
1	Ap, Ci, Of, Er, Ge, Va, Am
1	Ap, Er, Va, Am
1	Ap, Va, Am, Ce
2	Ap, Ge, Am
1	Ap, Er, Va
1	Ap, Ci
1	Ap, Gen, Ce

185 Ap = ampicillin, Ci = ciprofloxacin, Of = ofloxacin, Er = erythromycin, Ge = gentamycin,
186 Va = vancomycin, Am = amoxicillin, Ce = ceftriaxone

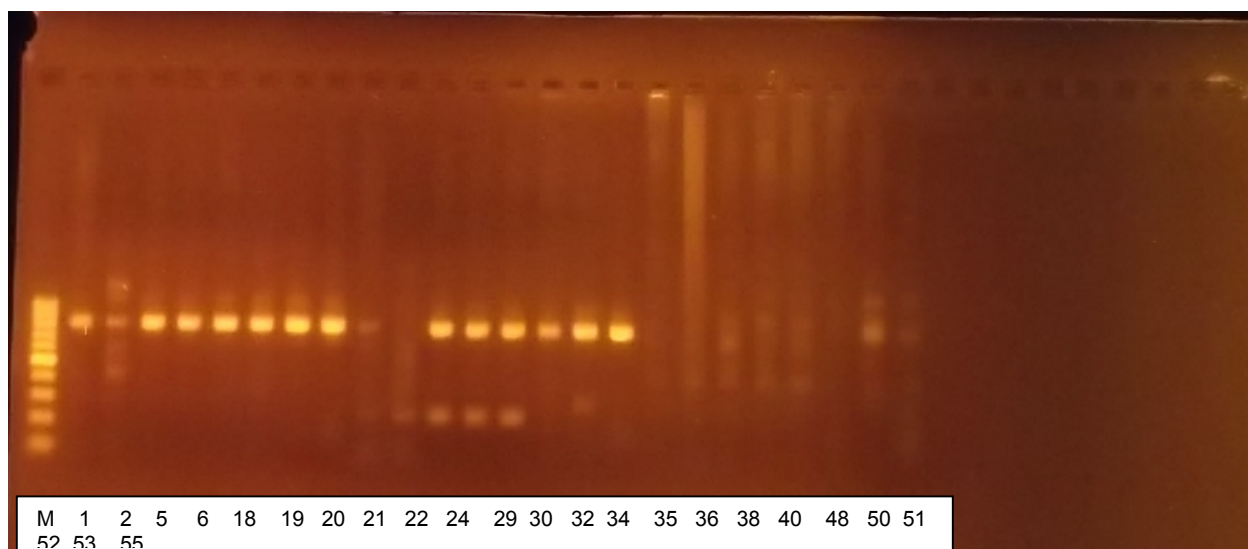
187
188 A total of 24 isolates representing 47.1% of the total MRSA isolates were found to possess
189 plasmid. Single plasmid isolates were 14(27.5%), double plasmid isolates were 6(11.8%)
190 while tripple plasmid isolates were 4(7.8%). Twenty seven isolates were found to have no
191 plasmid (table 2). The 24 isolates containing plasmids were categorized into 14 groups
192 based on their resistogram. Plasmid profile showed greater similarity between isolates (10
193 profiles) than antibiotic resistance pattern which showed a higher disparity (14 patterns).

194
195 Table 2: Plasmid profile of MRSA isolated from healthy individuals
196 n = 51

Plasmid profile group	Plasmid profile	No of isolates (%)
0	-	27(52.9)
1	3000	2(3.9)
2	500, 1000	1(2.0)
3	4000	7(13.7)
4	500	2(3.9)
5	300	3(5.9)
6	500, 4000	3(5.9)
7	300, 4000	1(2.0)
8	300, 500, 700	4(7.8)
9	1000, 1500	1(2.0)

197
198 In characterizing MRSA isolates epidemiologically, antibiotic profile is poorly discriminatory,
199 type specific antisera for serologic tests are expensive, phage typing are often non-
200 reproducible and some strains are non-typeable.

201 Restriction endonuclease analysis of plasmid DNA resolved all the isolates in this study into
202 10 EcoRI profiles (figure 1 and table 2).
203
204
205



206
207 Figure 1: Plasmid profile of MRSA after the EcoRI restriction digestion reaction set up with
208 the selected 24 plasmid DNA.

209 Even though studies have demonstrated the stability and diversity of restriction enzyme
210 profile of plasmid DNA by finding very few strains of MRSA obtained from various sources
211 and locations to lack plasmid entirely [27], plasmid profile appeared to be of low
212 discriminatory power in the investigation of MRSA epidemiology because of the non-
213 detection of plasmid in majority of the isolates of MRSA in this study. This could be due to
214 the fact that most isolates have low copy number plasmids which were not detected.
215 Plasmid profile has been reported to be useful in tracing the epidemiology of antibiotic
216 resistance [19]. However, in this study, resistance to various antimicrobial agents was not
217 consistent with the presence of plasmids. This was because no particular molecular size
218 plasmid could be associated with any particular antimicrobial resistance patterns. Resistance
219 was observed in isolates with various molecular size plasmids as well as in those that had
220 no plasmids. The reason for this could not be explained but could be due to various attitudes
221 of the participants towards use of antibiotics. Nonetheless, while there existed in this study
222 isolates with same resistance pattern but with different plasmid profile, 2 pairs of isolates
223 with the same plasmid profile also have similar (almost the same) resistance pattern (table
224 3).
225

226 Table 3: Plasmid profile and resistogram of plasmid DNA containing isolates
227 n = 24

Serial number	Lab number	Resistance pattern	Plasmid profile
1	1=E ₁	Ap, Ci, Er, Ge, Va, Am, Ce	3000
2	2= E ₃	Ap, Of, Er, Va, Am, Ce	500, 1000
3	5= E ₆	Ap, Ge, Am	4000
4	6= E ₇	Ap, Am	4000
5	18= E _{12b}	Ap, Er, Va	4000
6	19= E _{17a}	Ap, Am	4000

7	20= E ₁₇ b	Ap	4000
8	21= E ₁₈	Ap	4000
9	22= E ₁₉	Ap, Ci	500
10	24= N ₁	Ap, Va, Am	300
11	29= N ₈	Ap, Am	500, 4000
12	30= N ₉	Ap, Am	500, 4000
13	32= N ₁₁	Ap, Ci, Of, Er, Ge, Va, Am, Ce	500, 4000
14	34= N ₁₂	Ap, Am, Ce	3000
15	35= N ₁₃	Ap, Am	300, 4000
16	36= N ₁₅	Ap, Ge	4000
17	38= N ₁₆ b	Ap, Am	300
18	40= N ₁₈	Ap, Am	500
19	48= U ₁₄	Ap	300, 500, 700
20	50= U ₁₆ a	Ap, Am	300, 500, 700
21	51= U ₁₆ b	Ap	300, 500, 700
22	52 U ₁₀ a	Ap, Va, Am	300
23	53= U ₁₀ b	Ap, Va	1000, 1500
24	55= U ₉ a	Ap, Ge, Ce	300, 500, 700

Ap = ampicillin, Ci = ciprofloxacin, Of = ofloxacin, Er = erythromycin, Ge = gentamycin, Va = vancomycin, Am = amoxicillin, Ce = ceftriaxone.

The resistance of MRSA isolates to various antimicrobials may be located either on chromosomes, plasmids or transposons. Methicillin resistance gene (*mec4916*) has a chromosomal locus, and is probably maintained on mobile genetic element while tetracycline resistance observed in *S. aureus* strain DU4916 was reported to be encoded by a 4.3 kb plasmid, and in some strains resistance was found to be encoded by a chromosomal gene [19].

4. CONCLUSION

In conclusion, this study showed that the genetic basis for antibiotic resistance in the study area is not entirely plasmid mediated. Plasmid profile analysis in conjunction with the antibiogram is valuable in differentiating various strains of community acquired MRSA.

CONSENT

All authors declare that verbal informed consent was obtained from the participants for publication of this result.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abdalla NM, Haimour WO, Osman AA, Abdulaziz H. Assessment of the Multifactorial Effect on Antimicrobial Sensitivity in Positive *Staphylococcus*

- 257 *aureus* Clinical Isolates from Assir Region, Saudi Arabia. Journal of Medicine.
258 2012; 13(2):152-159.
259
- 260 2. Akano SO, Daini OA, Ojo MO, Smith SI, Akinside KA. Comparative analysis of
261 antibiotic resistance and r-plasmids OF *Staphylococcus aureus* isolates from
262 human and dog samples. African Journal of Clinical and Experimental
263 Microbiology. 2009; 10(3):136-143.
- 264 3. Al-Anazi AR. Prevalence of *Methicillin-Resistant Staphylococcus aureus* in a
265 teaching hospital in Riyadh, Saudi Arabia. Biomedical Research. 2009; 20(1):7-
266 14.
- 267 4. Al-Hamdani MA, Hamad IG. Study of plasmid profile, susceptibility patterns of
268 clinical *Staphylococcus aureus* isolated from patients with otitis media in Basrah.
269 Journal of Basrah Researches ((Sciences)). 2012; 38(1):79-89.
- 270 5. Al-Jumaily EF, Mohamed DA, Khanaka HH. Molecular epidemiology and
271 Antibiotic susceptibility patterns of clinical strains of methicillin resistant
272 *Staphylococcus aureus* (MRSA) in Sulaimani city-Iraq. Global Advanced
273 Research Journal of Microbiology, 2012; 1(6):081-089.
- 274 6. Al-Mohana AM, Al-Charrakh AH, Nasir FH, Al-Kudhairy MK. Community-
275 acquired methicillin-resistant *Staphylococcus aureus* carrying *mecA* and Panton-
276 Valentine leukocidin (PVL) genes isolated from the holy shrine in Najaf, Iraq.
277 Journal of Bacteriology Research. 2012; 4(2):15-23.
- 278 7. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for
279 Antimicrobial Susceptibility Testing, Seventeenth International Supplement,
280 CLSI document M100-S17. Vol. 27-1 [ISBN 1-56238-625-5], PA, USA. 2007.
281
- 282 8. Clinical and Laboratory Standards Institute (CLSI). Performance standards for
283 antimicrobial susceptibility testing. Approved standard M100-S20. Vol. 30, No. 1.
284 National Committee for Clinical Laboratory Standards, Wayne, PA. USA. 2010.
285
- 286 9. Corvaglia AR, François P, Hernandez D, Perrona K, Lindera P, Schrenzel J. A
287 type III-like restriction endonuclease functions as a major barrier to horizontal
288 gene transfer in clinical *Staphylococcus aureus* strains. Preceedings of the
289 National Academy of Sciences. 2010; 107(27):11954–11958.
290
- 291 10. Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M, Bhat KH, Dar MJ,
292 Ahmed N, Ahmad S. Molecular epidemiology of clinical and carrier strains of
293 methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of
294 north India. 2006. Accessed February, 2013. Available: <http://www.Ana-clinmicrob.com/content/5/1/22>.
295
296
- 297 11. den Heijer CDJ, van Bijnen EME, Paget WJ, Pringle M, Goossens H,
298 Bruggeman CA, Schellevis F, G, Stobberingh GE, the APRES Study Team.
299 Prevalence and resistance of commensal *Staphylococcus aureus*, including

- 300 meticillin-resistant *S aureus*, in nine European countries: a cross-sectional
301 study. *Lancet Infectious Diseases*. 2013; 13:409–415.
302
- 303 12. Haque ME, Shahriar M, Haq A, Gomes BC, Hossain MM, Razzak MA, Mazid
304 MA. Prevalence of β -lactamase-producing and non-producing methicillin
305 resistant *Staphylococcus aureus* in clinical samples in Bangladesh Prevalence
306 of β -lactamase-producing and non-producing methicillin resistant
307 *Staphylococcus aureus* in clinical samples in Bangladesh. *Journal of*
308 *Microbiology and Antimicrobials*. 2011; 3(5):112-118.
309
- 310 13. Hena JV, Sudha SS. RFLP Analysis of Clinical MRSA Isolates. *International*
311 *Journal of Pharmacy and Biological Sciences*. 2011; 2(1):637-645.
312
- 313 14. Kennedy AD, Porcella SP, Martens C, Whitney AR, Braughton KR, Chen L,
314 Craig CT, Tenover FC, Kreiswirth BN, Musser JM, DeLeo FR. Complete
315 Nucleotide Sequence Analysis of Plasmids in Strains of *S. aureus* Clone
316 USA300 Reveals a High Level of Identity among Isolates with Closely Related
317 Core Genome Sequences. *Journal of Clinical Microbiology*. 2010; 48(12):4504–
318 4511.
319
- 320 15. Khadri H, Alzohairy M. Prevalence and antibiotic susceptibility pattern of
321 methicillin-resistant and coagulase-negative staphylococci in a tertiary care
322 hospital in India. *International Journal of Medicine and Medical Sciences*. 2010;
323 2(4):116-120.
324
- 325 16. McDougal LK, Fosheim GE, Nicholson A, Bulens SN, Limbago BM, Shearer
326 JES, Summers AO, Patel JB. Emergence of Resistance among USA300
327 Methicillin-Resistant *Staphylococcus aureus* Isolates Causing Invasive Disease
328 in the United States. *Antimicrobial Agents and Chemotherapy*. 2010;
329 54(9):3804–3811.
- 330 17. Moussa IM, Al-Qahtani AA, Gassem MA, Ashgan MH, Ismail DK, Ghazy AI,
331 Shibl AM. Pulsed-field gel electrophoresis (PFGE) as an epidemiological
332 marker for typing of methicillin-resistant *Staphylococcus aureus* recovered from
333 KSA. *African Journal of Microbiology Research*. 2011; 5(12):1492-1499.
- 334 18. Okwu M, Bamgbala S, Aborisade W. Prevalence of Nasal Carriage of
335 Community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA)
336 among Healthy Primary School Children in Okada, Nigeria. *Journal of Natural*
337 *Sciences Research*. 2010; 12(4):61-65.
- 338 19. Olowe OA, Eniola KIT, Olowe RA, Olayemi AB. Antimicrobial Susceptibility and
339 Beta-lactamase detection of MRSA in Osogbo, SW Nigeria. *Nature and Science*.
340 2007; 5(3):44-48.
- 341 20. Ombui JN, Kimotho AM, Nduhiu JG. Antimicrobial resistance patterns and
342 plasmid profiles of *Staphylococcus aureus* isolated from milk and meat. *East*
343 *African Medical Journal*. 2000; 77(9):463-465.
344

- 345 21. Orji I, Nworie A, Eze UA, Agberotimi IO, Okereke EC, Azi SO. The prevalence
346 and antimicrobial susceptibility profile of methicillin-resistant *Staphylococcus*
347 *aureus* isolates from clinical specimens in a tertiary hospital, south east Nigeria.
348 Continental Journal of Pharmaceutical Sciences. 2012; 6(1): 23 – 29.
349
- 350 22. Perveen I, Majid A, Knawal S, Naz I, Sehar S, Ahmed S, Raza MA. Prevalence
351 and Antimicrobial Susceptibility Pattern of Methicillin-Resistant *Staphylococcus*
352 *aureus* and Coagulase-Negative Staphylococci in Rawalpindi, Pakistan. British
353 Journal of Medicine & Medical Research. 2013; 3(1):198-209.
354
- 355 23. Prakash M, Rajasekar K, Karmegam N. Prevalence of Methicillin-Resistant
356 *Staphylococcus aureus* in Clinical Samples Collected from Kanchipuram Town,
357 Tamil Nadu, South India. Journal of Applied Sciences Research. 2007;
358 3(12):1705-1709.
- 359 24. Saba AJ, Budimir A, Nashev D, Sá-Leão R, van Dijl JM, Laurent F, Grundmann
360 H, Friedrich AW, ESCMID Study Group of Epidemiological Markers (ESGEM).
361 Overview of molecular typing methods for outbreak detection and
362 epidemiological surveillance. 2013. Accessed February, 2013. Available:
363 <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20380>.
- 364 25. Shearer JES, Wireman J, Hostetler J, Forberger H, Borman J, Gill J, Sanchez S,
365 Mankin A, LaMarre J, Lindsay JA, Bayles J, Nicholson A, O'Brien F, Jensen
366 SO, Firth N, Skurray RA, Summers AO. Major Families of Multiresistant
367 Plasmids from Geographically and Epidemiologically Diverse Staphylococci.
368 Gens/Genomes/Genetics. 2011; 1:581-591.
369
- 370 26. Shen H, Akoda E, Zhang K. Methicillin-Resistant *Staphylococcus aureus*
371 Carriage among Students at a Historically Black University: A Case Study.
372 International Journal of Microbiology, Article ID 979734. 2012. Accessed
373 February, 2013. Available: <http://dx.doi.org/10.1155/2013/979734>.
374
- 375 27. Taiwo SS. Methicillin Resistance in *Staphylococcus aureus*: A Review of the
376 Molecular Epidemiology, Clinical Significance and Laboratory Detection
377 Methods. West African Journal of Medicine. 2009; 28(5):281-290.
378
- 379 28. Taiwo SS, Bamidele M, Omonigbehin EA, Akinsinde KA, Smith SI, Onile BA,
380 Olowe AO. Molecular epidemiology of methicillin resistance *S. aureus* in Ilorin,
381 Nigeria. West African Journal of Medicine. 2005; 24(2):100-106.
- 382 29. van Leeuwen WB. Molecular Diagnostics in Clinical Microbiology. Iranian
383 Journal of Microbiology. 2009; 1(2):5 – 20.
- 384 30. Yamamoto T, Dohmae S, Saito K, Otsuka T, Takano T, Chiba M, Fujikawa K,
385 Tanaka M. Molecular Characteristics and In Vitro Susceptibility to Antimicrobial
386 Agents, Including the Des-Fluoro (6) Quinolone DX-619, of Pantone-Valentine
387 Leucocidin-Positive Methicillin-Resistant *Staphylococcus aureus* Isolates from
388 the Community and Hospitals. Antimicrobial Agents and Chemotherapy. 2006;
389 50(12):4077–4086.