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Research article

Prevalence and comparison of three methods for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in tertiary health institutions in Nigeria.

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Abstract

Different epidemiological factors such as geographical and health system capacity in running infection control programmes have roles in variability of prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA). The objectives of this study were to determine the prevalence and compare three methods for the detection of clinical isolates of MRSA in Jos University Teaching Hospital (JUTH), University of Nigeria Teaching Hospital (UNTH),

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Cherith Diagnostic Laboratory (CDL) and University of Benin Teaching Hospital (UBTH). A total of 290 *S. aureus* isolates obtained from urine, semen, wound, ear, eye and high vaginal swab (HVS) samples were collected from patients admitted in the hospitals. The isolates were screened for MRSA by oxacillin screen agar test which was compared with methicillin disc diffusion and Polymerase Chain Reaction (PCR) mecA gene detection techniques. There was no significant difference ($p > 0.05$) between the three methods. At JUTH, the prevalence of MRSA was 33.3%; 17.5% from males and 15.9% from females. At UNTH, prevalence was 42.7%; 24% from males and 18.7% from females. CDL had MRSA prevalence of 40.3%; 16.7% from males and 23.6% from females. At UBTH, it was 35% with 11.3% from the males and 23.8% from females. There were no significant differences between the sexes and the health institutions. At JUTH, the prevalence of MRSA was highest in wound samples with 12.7%, followed by urine with 9.5%. At UNTH, the highest prevalence was in urine samples with 21.3%, followed by wound with 13.3%. At CDL and UBTH, MRSA prevalence was highest in HVS with 16.7% and 15% respectively followed by urine with 12.5% and 11.3% respectively. There was no significant difference between the sample types. In conclusion, there is an urgent need for prompt detection, regular effective surveillance of MRSA in Nigerian hospitals to aid in the effectual treatment of MRSA infections.

Key words: methicillin-resistant *S. aureus* (MRSA), prevalence, polymerase chain reaction, mecA gene.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of the bacterium *Staphylococcus aureus.* It is characterized by antibiotic resistant to methicillin and many other chemotherapeutic agents (1). MRSA can cause the same types of infections as *S. aureus* isolates such as: skin and soft tissue infections including impetigo, folliculitis, furunculosis, cellulitis, abscesses and wound infections (2, 3). MRSA can also cause invasive infections such as: pneumonia, endocarditis, septic arthritis, meningitis, osteomyelitis, septicemia, toxic shock and staphylococcal scalded skin syndromes in infants and adults (4, 5, 2, 6). Patients with compromised immune systems are at greater risk of symptomatic secondary infections.

MRSA infections have now become a major public health concern and its prevalence is increasing globally (7). Available literatures indicated the wide prevalence rate of MRSA in various countries as: 1-5% in Northern Europe, 5-30% in Southern Europe, 5-40% in Asian countries and 10-50% in USA and UK (8). The prevalence rate ranged from 2% in the Netherlands and Switzerland to 70% in Japan and Hong-Kong (9, 10). A prevalence rate of 21-30% in Nigeria was reported by Gorwitz, *et al.,* (11). Different epidemiological factors such as geographical and health system capacity in running infection control programmes have roles in variability of prevalence of MRSA (12).

Rapid and reliable identification of MRSA is very important in order to choose appropriate therapy, to prevent unnecessary use of glycopeptides antibiotics and to take necessary measures for infection control. Also, correct identification helps to avoid economic loss caused by unnecessary infection control precautions (13). Studies that assess phenotypic and genotypic methods for the detection/identification of MRSA are extensive in literature and different recommendations have been presented regarding the most reliable method for routine use (13).

Jos University Teaching Hospital (JUTH) is located in Jos (capital of Plateau), north-central zone of Nigeria. It has health facilities of 750-1,000. Plateau state has an estimated population of about 3.2 million with coordinates $9^0$56´ N, $8^0$53´ E. Jos enjoys a more temperate climate than most parts of Nigeria. Average monthly temperatures range from 21-25^{0}C and from mid-November to late January. Night time temperatures drop as low as 11^0C (14).

University of Nigeria Teaching Hospital (UNTH) is located in Enugu, south-east zone of Nigeria. It has health facilities of 500-700. Enugu state has an estimated population of 3.5 million people with coordinates $6^0$30´ N,

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$7^0$30´E. It lies in the rain forest zone with two major seasons (raining and dry). Temperature is about 28-30^{0}C (14, 15).

Cherith Diagnostic laboratory (CDL) is a major laboratory servicing the neighbouring hospitals in the environs. CDL is situated in Lagos state which is located in the south-west zone of Nigeria with coordinates $6^0$27´11´´ N $3^0$23´ 45´´ E. The population in official record, according to Nigerian census figures of 2006 was 9,013,534. It has health facilities of 1,000-1,500 (14).

University of Benin Teaching Hospital (UBTH) is located in Benin City, Edo state, south-south, Nigeria. It has coordinates $6^0$30´N $6^0$00´E. In 2006, the population size was estimated as 3.2 million with health facilities of 500-750 (14).

Since clinically significant MRSA is being isolated with greater frequency in many countries, this study was conducted to investigate the prevalence and compare three methods for the detection of clinical isolates of MRSA in JUTH, north-central; UNTH, south-east; CDL, south-west and UBTH, south-south of Nigeria.

Materials and Methods

Media and Antibiotics

The media used were: mannitol salt agar (Chapman medium USP. Eur Pharm), Mueller-Hinton agar, blood agar, nutrient broth and agar from Maharashtra, India. Antibiotics used were oxacillin (Odypharm, UK) and methicillin (Oxoid, UK).

Sources of clinical isolates

All the clinical isolates of *S. aureus* used in this study were obtained from: urine, semen, wound, eye, ear and high vaginal swab samples collected from patients admitted in JUTH, UNTH, UBTH and samples submitted to CDL.The collections of samples were according to Cheesbrough methods (16). The samples were labeled, packaged, transported to the Microbiology laboratory and cultured within 1-3 hours of collection.

Identification procedures

The isolates were identified using standard microbiological techniques which included colonial morphology, Gram's stain reactions and biochemical tests (16). Isolates that were: Gram's positive and coccoid in clusters, catalase, coagulase, deoxyribonuclease and mannitol fermentation positive were considered as *S. aureus* in this study.

Tests for MRSA isolates

S. aureus isolates were tested for susceptibility to oxacillin using oxacillin screen agar test as described by Kumurya, *et al.*, (17). Oxacillin resistant isolates were recorded as MRSA and susceptible isolates as MSSA (methicillin-susceptible *S. aureus*).

Test for methicillin susceptibility was by using modified Kirby-Bauer disc diffusion technique (16). Inhibition zone diameters of the isolates were measured in millimeters with a ruler and interpreted according to the interpretative chart of Clinical and Laboratory Standards Institute (18). The tests were done in triplicates and the control strain *S. aureus* NCIB 8588 was included.

Molecular detection of MRSA isolates using Polymerase chain reaction (PCR).

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Pure cultures of each *S. aureus* isolate were grown in 2 ml of nutrient broth for 18-24 hours at 37^0C, adjusted to 0.5 McFarland and centrifuged at 5,000 x g for 10 minutes. Total DNA was extracted from the pellets using the QIAamp DNA minikit (Qiagen, USA) according to manufacturer's instructions. The final elution volume was used for amplification. A volume of 25 µl PCR reaction mixture consisting of 10 µl of PCR mix (100 mM Tris-HCl, 500 mM KCl, 15mM $MgCl_2$, 0.01% gelatin, 10 mM dNTP, Taq polymerase), primers (1 µl each), double distilled water (10 µl), and DNA template (3 µl) was used for PCR. A 408-bp fragment of the mecA gene was amplified using the primers; mecA-F: 5' CAA GAT ATG AAG TGG TAA ATG GT-3' and mecA-R: 5' TTT ACG ACT TGT TGC ATA CCA TC-3' (19). *S. aureus* NCIB 8588 was used as a negative control. Amplification was performed in a DNA thermal cycler (Peltier, USA) beginning with an initial denaturation step at 94^0C for 5 minutes, followed by 30 cycles of denaturation at 94^0C for 30 seconds, annealing at 55^0C for 45 seconds, extension at 72^0C for 1 minute and final extension at 72^0C for 5 minutes, followed by a hold at 4^0C. The amplified product (10 µl) was electrophoresied on a 2% Tris-acetate-EDTA agarose gel at 90 volts for 1 hour (after staining with ethidium bromide) (2 µg/ml) and visualized under UV transilluminator (Alpha Innotech Corporation) (13, 20).

Statistical analysis

Frequencies were obtained, percentages were calculated for the study variables, parametric and non-parametric T-test, analysis of variance (ANOVA) and Duncan's Multiple Range (DMR) test (used to establish significant difference) were applied. Statistical Package for Social Sciences (SPSS), version 20.0 was used.

Results and discussion

A total of 290 *S. aureus* isolates obtained from urine, semen, wound, eye, ear and high vaginal swab samples were collected from patients admitted in JUTH, UNTH, UBTH and samples submitted to CDL, Nigeria. Table 1 shows the prevalence values of MRSA isolates with respect to sex in JUTH, UNTH, CDL and UBTH, Nigeria. In a total of 63 *S. aureus* isolates obtained from JUTH, 46% (29/63) of the isolates were from males while 54% (34/63) from females. The prevalence of MRSA was 33.3% (21/63); 17.5% (11/63) from males and 15.9% (10/63) from females. At UNTH, of the 75 *S. aureus* isolates, 45.3% (34/75) were from males while 54.7% (41/75) from females. The prevalence of MRSA was 42.7% (32/75), 24% (18/75) from males and 18.7% (14/75) from females. *S. aureus* isolates from CDL were 72; 41.7% (30/72) from males and 58.3% (42/72) from females. MRSA prevalence was 40.3% (29/72); 16.7% (12/72) from males and 23.6% (17/72) from females. Of the 80 *S. aureus* isolates from UBTH, 37.5% (30/80) were from males and 62.5% (50/80) from females. MRSA prevalence was 35% (28/80); 11.3% (9/80) from males and 23.8% (19/80) from females. There was no significant difference ($p > 0.05$) between the sexes and the health institutions.

Table 2 shows the prevalence/distribution of MRSA isolates in clinical samples obtained from JUTH, UNTH, CDL and UBTH, Nigeria. The samples collected were: urine, semen, wound, eye, ear and high vaginal swabs (HVS). At JUTH, urine samples were 36.5% (23/63); wound, 30% (17/63); HVS, 23.8% (15/63) and semen, 12.7% (8/63). MRSA was highest in wound samples with 12.7% (8/63), followed by urine with 9.5% (6/63), HVS with 6.3% (4/63) and least in semen with 4.8% (3/63). At UNTH, urine samples were 44% (33/75); wound, 24% (18/75); HVS, 25.3% (19/75) and semen, 6.7% (5/75). MRSA was highest in urine samples with 21.3% (16/75), followed by wound with 13.3% (10/75), HVS with 5.3% (4/75) and least in semen with 2.7% (2/75). At CDL, urine samples were 36.1% (26/72); HVS, 43.1% (31/72) and semen, 20.8% (15/72). MRSA was highest in HVS with16.7% (12/72), followed by urine with 12.5% (9/72) and least in semen with 11.1% (8/72). At UBTH, urine samples were 36.3% (29/80); wound, 13.8% (11/80); HVS, 38.8% (31/80) and eye/ear swabs, 11.3% (9/80). MRSA was highest in HVS with 15% (12/80), followed by urine with 11.3% (9/80); wound, 6.3% (5/80) and least in eye/ear swabs with 2.5% (2/80). There was no significant difference ($p > 0.05$) between the sample types.

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Of the 42 *S. aureus* isolates randomly screened for mecA gene using polymerase chain reaction (PCR), 26.2% were mecA positive while 73.8% were negative. For oxacillin screen agar test, 28.6% were oxacillin positive (MRSA) while 71.4% were negative (MSSA) (table 3). There was no significant difference ($p > 0.05$) between the two methods for the detection of MRSA.

In addition, eleven *S. aureus* isolates were randomly selected and screened for MRSA using oxacillin screen agar test, methicillin disc diffusion technique and PCR methods. Detection of mecA gene using PCR showed that 7 of them were mecA gene positive while 4 were negative. With oxacillin screen agar test, 6 were oxacillin positive and 5 negative. Only 9 of the isolates were screened with methicillin discs. Four were resistant while 5 were sensitive (table 4). There was no significant difference ($p > 0.05$) between the three screening methods.

Figure1 depicts PCR result to detect mecA gene (408-bp). *S. aureus* isolates 27J, 22J, 227L, 20D and 53B were mecA positive while the other isolates were negative.

MRSA is a strain of *S. aureus* which was first reported in 1961 soon after methicillin was introduced and it has since emerged as an important pathogen in human medicine (21, 22). MRSA infections have now become a major public health concern and its prevalence is increasing globally (7). The prevalence of MRSA in JUTH, North-Central of Nigeria was found to be lower in this study than the 43% previously reported by Ikeh, (23) at the same hospital. The reason for the disparity in values cannot be ascertained in this study. However, comparative clinical reports from other studies from the Northern zones of Nigeria showed that the value in this present study was higher than the 28.6% reported by Nwankwo, *et al.,* (24) from Aminu Kano Teaching hospital, Kano (North-West) and the average of 12.5% in six hospitals reported by Okon, *et al.,* (25) in North-East Nigeria. The prevalence of 42.7% MRSA from UNTH, South-East of Nigeria in this present study is much lower than the 77% reported by Orji, *et al.,* (26) in the same zone at Abakaliki, Ebonyi State University Teaching hospital, Nigeria. The report from a previous study in the South-West zone of Nigeria showed that the prevalence of 40.3% MRSA recorded in CDL (South-West) in this present study was higher than the 22.2% reported by Alli, *et al.,* (27). At UBTH, South-South, the prevalence recorded in this study was lower than the 79% MRSA earlier reported by Onemu and Ophori, (28) and higher than the 11% reported by Obasuyi, (29) at the same hospital. These observations from the present study are in agreement with previous studies that reported that the prevalence of MRSA in Nigeria usually varies between 34.7% and 71.2% (30, 31, 32). The variation in MRSA prevalence from different zones of Nigeria could be due to differential clonal expansion and drug pressure in each community. In addition, MRSA prevalence varies greatly with geographical location, types of hospitals, method of detection employed and studied population (32). However, phenotypic MRSA screening tests should be done with utmost care and at least in triplicates for accurate results. This is to avoid wide discrepancies in prevalence values in the same locations. The prevalence of MRSA in various geographical locations in Nigeria seems not to be dependent on the temperatures of the locations. The lowest prevalence of 33.3% observed in North-Central (with an average temperature of 21-25^{0}C and night temperature of 11^{0}C) compared to South-West, South-East and South-South, Nigeria (with an average temperature of 28^{0}C-37.3^{0}C) was probably not due to the lower temperature in North-Central. This is because higher prevalence of MRSA has been reported in various locations (countries) with low temperatures (33). Also, prevalence values of 52% and above of MRSA have been reported in tertiary hospitals in US, Southern European countries, Asia and South America where temperatures can be as low as below freezing temperatures during winter. A progressive increase in the prevalence values of MRSA all over the world has been reported (33).

At JUTH and UNTH, the MRSA prevalence was higher in males than females while at CDL and UBTH, it was higher in females than males. However, statistical tests showed there was no significant difference between the sexes ($p > 0.05$). This is in agreement with the findings of Ankur, *et al.,* (34) and Okwu, *et al.,* (35) who reported that there was no significant difference in the carriage rates of MRSA isolates between male and female subjects.

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At North-Central (JUTH), the highest prevalence of MRSA was observed in wound samples while in urine samples at South-East (UNTH) and in HVS at South-West (CDL) and South-South (UBTH) in this study. However, there was no significant difference ($p > 0.05$) between sample types. Previous studies by Ikeh, (23) and Nwankwo, (24) reported that the highest rate of MRSA was found in samples from surgical wound infections while Orji, *et al.,* (26) reported that urine samples gave the highest rate of MRSA. Urinary tract infection accounts for the majority of hospital visits and it is the second most clinical indication for empirical antibiotic treatment in primary and secondary health-care (26). It has been reported that the majority of the empirically prescribed antibiotics do not correlate with results of subsequent culture and susceptibility and has been noted to be contributing to the high resistance of microorganisms to antimicrobial agents (26).

Methicillin resistance in *S. aureus* could either be due to the expression of mecA gene or the synthesis of methicillinase or to both factors. Detection of mecA gene by PCR methods is considered the gold standard for MRSA confirmation. However, it is not practical for routine use in clinical laboratories because it is time consuming and technically demanding (36). Kumurya, *et al.,* (17) reported that oxacillin screen agar test has been evaluated and recommended as an excellent alternative for the detection of MRSA isolates. The presence of resistance in *S. aureus* isolates on an oxacillin screen agar plate shows that the isolates are mecA positive (36). Therefore, oxacillin screen agar test was preferred for the screening of methicillin resistance in *S. aureus* isolates in this study since it compares favourably with PCR technique. The comparison of three MRSA screening methods (PCR for mecA detection, methicillin disc diffusion and oxacillin screen tests) showed no significant difference between the methods in this study. This is in agreement with the report of Kaya, *et al.,* (13). Therefore, the three methods were found to be satisfactory for MRSA detection.

Conclusion

The prevalence of clinical MRSA isolates in Nigerian tertiary hospitals is alarming; hence, there is an urgent need for the prompt detection and regular effective surveillance of MRSA in Nigerian health institutions. The establishment of an effective antibiotic therapy, reduction of the use of empirical treatment with broad-spectrum antibiotics, effective surveillance programme and emphasis on the importance of hand-washing as means of preventing the transmission of MRSA in hospitals will go a long way to control the scourge of MRSA in Nigerian hospitals.

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Table 1: Prevalence of MRSA isolates with respect to sex in JUTH, UNTH, CDL and UBTH, Nigeria.

	JUTH (North-Central) Number (Percentage)			UNTH (South-East) Number (Percentage)			CDL (South-West) Number (Percentage)			UBTH (South-South) Number (Percentage)		
Gender	**MRSA**	**MSSA**	**Total**	**MRSA**	**MSSA**	**Total**	**MRSA**	**MSSA**	**Total**	**MRSA**	**MSSA**	**Total**
Male	11(17.5%)	18(28.6%)	29(46%)	18(24%)	16(21.3%)	34(45.3%)	12(16.7%)	18(25%)	30(41.7%)	9(11.3%)	21(26.3%)	30(37.5%)
Female	10(15.9%)	24(38.1%)	34(54%)	14(18.7%)	27(36%)	41(54.7%)	17(23.6%)	25(34.7%)	42(58.3%)	19(23.8%)	31(38.8%)	50(62.5%)
Total	21(33.3%)	42(66.7%)	63(100%)	32(42.7%)	43(57.3%)	75(100%)	29(40.3%)	43(59.7%)	72(100%)	28(35%)	52(65%)	80(100%)

Key: JUTH- Jos University Teaching Hospital, UNTH- University of Nigeria Teaching Hospital, CDL- Cherith Diagnostic Laboratory, UBTH- University of Benin Teaching Hospital, MRSA- Methicillin-resistant *S. aureus*, MSSA- Methicillin-susceptible *S. aureus*

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Table 2: Prevalence/Distribution of MRSA in clinical samples from JUTH, UNTH, CDL and UBTH, Nigeria.

	JUTH (North-Central) Number (Percentage)			UNTH (South-East) Number (Percentage)			CDL (South-West) Number (Percentage)			UBTH (South-South) Number (Percentage)		
Sample	**MRSA**	**MSSA**	**Total**	**MRSA**	**MSSA**	**Total**	**MRSA**	**MSSA**	**Total**	**MRSA**	**MSSA**	**Total**
Urine	6(9.5%)	17(27%)	23(36.5%)	16(21.3%)	17(22.7%)	33(44%)	9(12.5%)	17(23.6%)	26(36.1%)	9(11.3%)	20(25%)	29(36.3%)
Wound	8(12.7%)	9(14.3%)	17(30%)	10(13.3%)	8(10.7%)	18(24%)	ND	ND	ND	5(6.3%)	6(7.5%)	11(13.8%)
Semen	3(4.8%)	5(7.9%)	8(12.7%)	2(2.7%)	3(4%)	5(6.7%)	8(11.1%)	7(9.7%)	15(20.8%)	ND	ND	ND
High vaginal swab	4(6.3%)	11(17.5%)	15(23.8%)	4(5.3%)	15(20%)	19(25.3%)	12(16.7%)	19(26.4%)	31(43.1%)	12(15%)	19(23.8%)	31(38.8%)
Eye/Ear swab	ND	ND	ND	ND	ND	ND	ND	ND	ND	2(2.5%)	7(8.8%)	9(11.3%)
Total	21(33.3%)	42(66.7%)	63(100%)	32(42.7%)	43(57.3%)	75(100%)	29(40.3%)	43(59.7%)	72(100%)	28(35.5%)	52(65%)	80(100%)

Key: JUTH- Jos University Teaching Hospital, UNTH- University of Nigeria Teaching Hospital, CDL- Cherith Diagnostic Laboratory, UBTH- University of Benin Teaching Hospital, MRSA- Methicillin-resistant *S. aureus*, MSSA- Methicillin-susceptible *S. aureus*

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Table 3: Comparative study of efficacy of polymerase chain reaction (PCR) for the detection of mecA gene and oxacillin screen test for identification of MRSA.

		MRSA/Oxacillin screen test		
PCR mecA	**No. of isolates (%)**	**MRSA**	**MSSA**	**Total**
Positive	11 (26.2%)	8	3	11
Negative	31 (73.8%)	4	27	31
Total	42 (100%)	12 (28.6%)	30 (71.4%)	42

Key: MRSA-Methicillin-resistant *S. aureus*, MSSA-Methicillin-susceptible *S. aureus*

Table 4: Comparison of three methods for the detection of MRSA isolates.

		Oxacillin screen test		Methicillin disc diffusion*	
PCR mecA gene	**No. of isolates**	MRSA	MSSA	Resistant	Sensitive
Positive	7	6	1	4	2
Negative	4	0	4	0	3
Total	11	6	5	4	5

Key: *2 *S. aureus* isolates not tested; MRSA- Methicillin-resistant *S. aureus*, MSSA-Methicillin-susceptible *S. aureus*

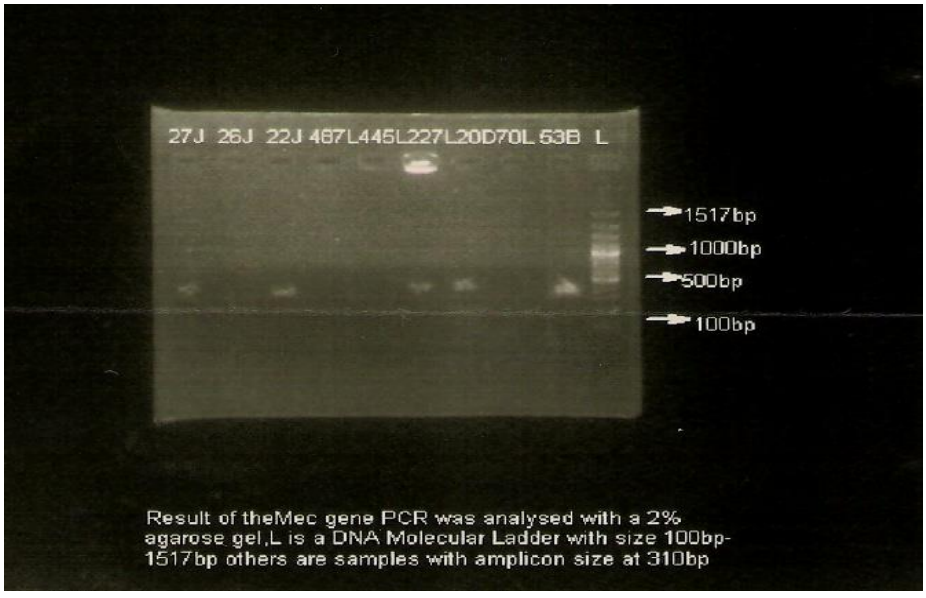

Figure 1: Lanes 27J – L depict PCR result to detect mecA gene (408 bp)

Lanes: 27J, 22J, 227L, 20D and 53B were mecA gene positive

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