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Isolation and Identification of *Staphylococcus aureus* from Iraqi Patient with Atopic Dermatitis in Baghdad Hospitals

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Abstract: Atopic dermatitis (AD) is one of the most chronic skin diseases that designated that redness, irritation and sever lesion. *S. aureus* has been tandemly detected in those lesions. The goal of the study was to characterize the aerobic and anaerobic microbiology of AD that had been secondary infected. Swab samples have been collected from patients diagnosed with AD. Specimens were examined microscopically and aerobically during cultivation using suitable culture media. The antibiotic susceptibility and the diagnosis of the bacterial isolates were confirmed using the VITEK 2 system. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for 2020. The PCR technique was applied to characterize the Methicillin-resistant *Staphylococcus aureus* (MRSA) pathogen and determine the prevalence of its most important virulence genes, including *16S rRNA* and the *SPA* gene. Eight different bacterial strains were tested from 200 AD patient swaps; all bacterial types isolated from eczematous lesions and nearly healthy areas were considered 80% *S. aureus* isolates and 20% other strains from eczematous lesions, while 40% *S. aureus* isolates and 60% other bacteria from nearly healthy areas. It was concluded all isolated *S. aureus* showed high resistance to the B-lactam group but showed variable susceptibility to other antibacterials used.

Keywords: Molecular diagnosis, PCR, *S. aureus*, Atopic Dermatitis, *PVL*.

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Introduction

Atopic dermatitis (AD) is the most common skin disease in infants and children. AD is a complex skin disease influenced by various factors such as genetic factors, skin barrier factors, predisposing factors, environmental factors, and precipitating factors. AD is usually characterized by an inflammatory reaction in the skin (1). This disease affects up to 15–20% of children and 7–10% of adults in developed countries (2). However, skin conditions or infections can be caused by an imbalance in host-microbe interactions that is influenced by endogenous (such as age or genetic variation) or exogenous (such as antibiotics or soap) variables (3). Dysbiosis refers to changes in the microbial community's makeup during illness (3). In atopic dermatitis (AD, i.e., eczema), patients are often highly colonized with *S. aureus* at lesional sites, and this bacterial "bloom" positively correlates with disease

severity (4). *Staphylococcus* represents the largest bacterial group on healthy skin and anterior nares (4). Moreover, the coagulase-negative staphylococci (CoNS) comprising *Staphylococcus epidermidis, Staphylococcus hominis,* and *Staphylococcus haemolyticus* are the most dominant species (4). CoNS are skin commensals that extensively interact with host epidermal and immune cells to maintain skin homeostasis and protect against opportunistic infections (5). Other microbes associated with inflammation and epithelial damage, such as *Clostridiodes difficile*, and *coliforms*, including pathogenic *Escherichia coli*, are increased in the gut microbiota of AD patients (6). *S. aureus* is present on the skin and mucous membranes of healthy individuals and remains in balance with the host; its overgrowth is most probably linked to the reduced number of skin microbiota representatives on affected skin, which normally inhibits the activity of this bacterium. *Staphylococcus aureus* has a major role in different types of eye infections such as conjunctivitis, keratitis, and endophthalmitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) was almost restricted to hospitals, but its prevalence has increased in people outside hospitals (7). Genes considered essential for an organism's survival are known as essential genes, *16S rRNA* gene sequence has been by far the most widely used housekeeping genetic marker in studies of bacterial phylogeny and taxonomy for a number of reasons, including its prevalence in nearly all bacteria and frequent existence as a multigene family, or operons (7). The *16S rRNA* gene is large enough for informatics uses, and its function has not changed over time, indicating that random sequence changes are a more accurate measure of time (evolution), the cell wall of *Staphylococcus aureus* has protein A, which can bind to the Fc portion of IgG, this protein A is encoded by the surface protein A of the *Staphylococcus aureus (spa)* gene, which contains a highly polymorphic sequence composed of repeats of 24-bp (7). Therefore, this present study aimed to identify and diagnose the prevalence of *Staphylococcus aureus* isolated from Iraqi atopic dermatitis patients in Baghdad hospitals.

Material and methods

Patients and control

A total of 100 individuals suffering from atopic dermatitis syndrome in various age groups and both sexes were included in this study. The patients attended the outpatient dermatology department of three main hospitals in Baghdad (outpatient-based study), and the diagnosis was performed by a dermatologist in the dermatologic clinic of the three hospitals. The study was carried out during the period from July 2022 to March 2023. Using Brain Heart Infusion Broth (BHIB) as the transport medium for all isolates, from each patient, two swabs were taken, one from an AD lesion skin and the other from a non-lesion skin.

Isolation and diagnosis

The isolates were morphologically diagnosed using colony morphology tests, such as the shape of the colonies and the formation of pigments on different agar media were applied for conformation. The antibiotic susceptibility test (AST) was achieved in accordance with the conformation of the biochemical diagnosis via VITEK 2 (BioMérieux Marcy-l'Étoile, France) towards 20 antimicrobial agents Goerke and Campana (2000). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for 2020.

Specific diagnosis for *S. aureus* was molecular diagnosis as follow

A: DNA was extracted using the Bacterial DNA Extraction Kit (New England Biolabs, UK). The detection of DNA integrity was performed using agarose gel electrophoresis (0.8%).

Table (1): Primers sequences used in this study

Target gene	Primer sequence (5'-3')		Product length (bp)	Reference
16SrRNA	**F**	5'- TGAAGGTCTTCGGATCGTAA -3'	550	This study
	R	5'- ATTAAACCACATGCTCCACC -3'		
SPA	**F**	5'- ACAGATGCAATACCTACACC-3'	614	This study
	R	5'-AAAGAACGCTCAACTGAAGA -3'		

Table (2): PCR thermal cycle

Name of gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension
16SrRNA	94°C per 5 min.	94°C per 30 sec.	50°C per 45 sec.	70°C per 30 sec.	70°C per 5 min.
SPA	94°C per 2 min.	94°C per 30 sec.	48°C per 45 sec.	70°C per 30 sec.	70°C per 7 min.

B: PCR amplification

S. aureus isolates that could be identified by their appearance were put through a PCR amplification of the species-specific *16SrRNA* and SPA genes, which should be about 550 and 614 base pairs, respectively. All reaction mixtures were set up to 25 µl reaction mixtures as follows: 12.5 µl of ready to use PCR master mix (New England Biolabs, UK), containing approximately 6 µl of template DNA and 1.5 µl (15 pmol) of each primer. The rest of the volume was completed with nuclease free water. The mixtures were subjected to the following thermal cycling parameters in a thermocycler (Applied Biosystem) shown in (Table 2). The PCR products were analysed on a 2% agarose gel, and the PCR products were compared for their molecular markers using a 100-bp DNA ladder. Two sets of primers for two genes were designed using (Geneious prime software) in this study are shown in (Table 1).

Statical analysis

The Statistical Analysis System-SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Results and discussion

Two hundred skin swabs were collected from patients admitted to the consulting clinic in the dermatology departments of three hospitals in Baghdad. The specimens were randomly collected and examined for the diagnosis of the secondary bacterial infection associated with eczema. About 96 samples were skin swabs from males, and the rest (104) were skin swabs from females of different ages from each health and lesion area per patient. In general, all 200 isolates from both areas were cultured on general and selective media after 24 hrs of incubation at 37 °C.

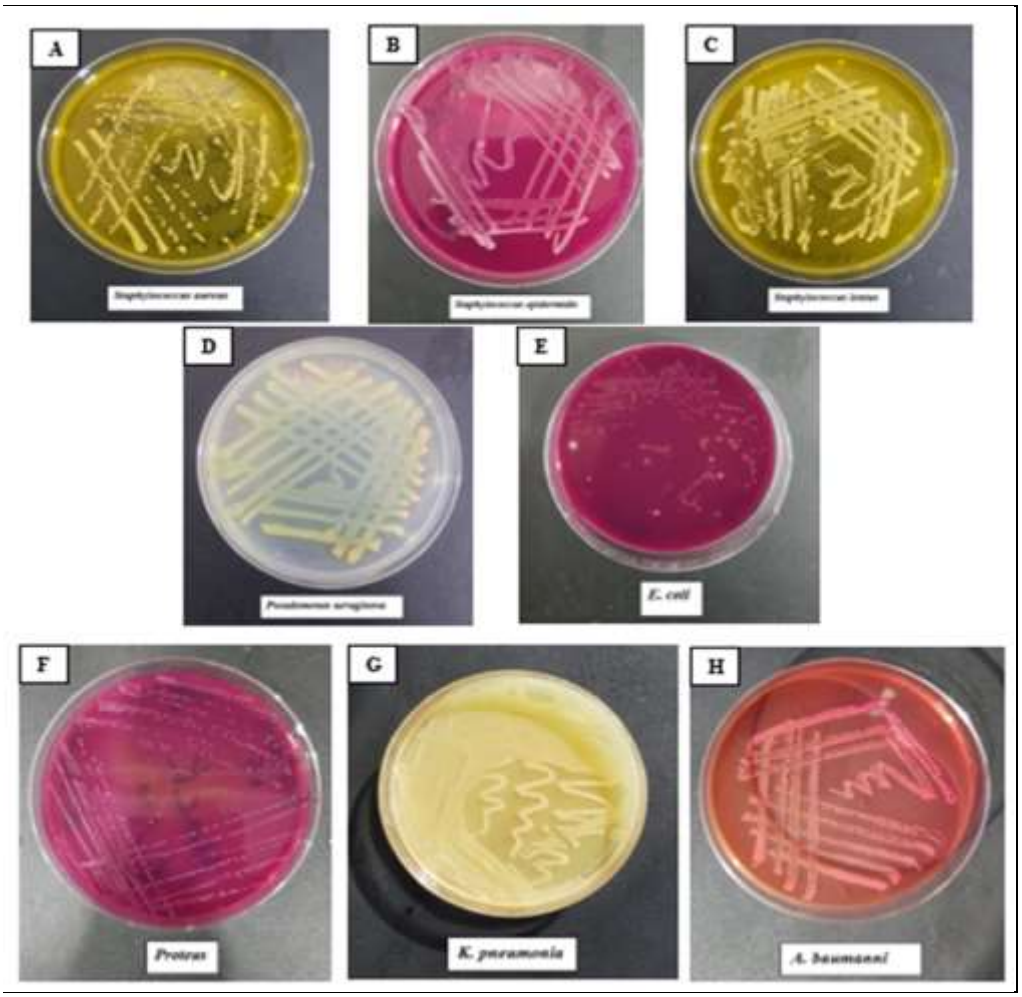

Figure (1): Illustrates all bacterial types as follow: (A) for *S. aureus*, (B) for *S. epidermidis*, (C) for *S. lentus*, (D) for *Pseudomonas aeruginosa*, (E) for *E. coli*, (F) for *Proteus Sp.*, (G) for *Klebsiella Sp.* and (H) for *Acinetobacter Sp.*

Table (3): biochemical andmolecular test for all bacterial isolates

Biochemical test	*S. aureus*	*S. epidermidis*	*S .lentus*	*Proteus Sp.*	*E .coli*	*K .pneumonia*	*P . aeruginosa*	*A . baumanni*
Oxidase	-	-	-	-	-	-	+	-
Catalase	+	+	+	+	+	+	+	+
Coagulase	+	-	-	+	+	+	-	+
Indole	-	-	-	-	+	-	+	+
Urease	+	+	+	+	+	+	+	+
Triple- Sugar Iron Agar H2S	-	+	-	+	-	-	-	-
Methyl Red	+	-	+	+	+	+	-	-
Voges proskauer	+	+	+	-	-	+	-	-
Simmons Citrate	+	-	-	+	-	+	+	-
16srRNA	+	+	+	-	-	-	-	-
SPA	+	+	+	-	-	-	-	-

The growing bacteria were identified biochemically (Table 3). These isolates were further identified by using the Vitek2 system, which is a new automatic system for the identification and susceptibility testing

of the most clinically important bacteria.

Eight different bacterial strains were identified in 200 AD patient swaps. All bacterial types isolated from eczematous lesions and nearly healthy areas were thought to be 80% S. aureus isolates and 20% other strains from eczematous lesions and 40% S. aureus isolates and 60% other bacteria from nearly healthy areas (Figures 1, 2, and 3). From the statical test (Table 4) refers to many bacteria including significant and non- significant bacteria for example significant bacteria such as *S. aureus, P. aeruginosa, E . coli* and *K . pneumonia* however, the other bacteria shown non- significant such as *S. epidermidis, S. lentus, Proteus Spp.* and *A . baumaani* , these might be explain as the non- significant bacteria as the normal flora in the skin. Bacterial densities on AD lesion skin are much higher than on healthy control skin, which is primarily because *S. aureus* abundances are significantly higher in AD lesion skin. *S.aureus* absolute abundances are similarly elevated in AD non-lesion skin, however not to the same extent as in lesion skin (11).

Previous studies in Iraq (8) detected another percentage in their study: *Staphylococcus epidermidis* (18, 40.9%), followed by *Staphylococcus aureus* (14, 31.8%), *Klebsiella pneumonia* (7, 15.9%), *Pseudomonas aeruginosa* (4, 9.1%), and *Proteus sp.* (1, 2.3%) from lesion areas of AD patients. Other studies found that *S. aureus* is a common colonization in AD lesion skin (10), with the ratio varying from lesion (70%) to non-lesion (39%) sites (9), which was closest to the study. *S. epidermidis* is thought to maintain skin microbiome balance by integrating innate immune pathways (9). *S. aureus* is becoming more prevalent in the skin's bacterial communities, which results in less variety when compared to the bacterial communities on healthy skin and increases in disease severity (11). *S. aureus*, a Gram-positive bacterium, is now acknowledged as an important triggering factor for the maintenance of skin inflammation and acute exacerbations of the genetically determined skin disease due to advances in our understanding of the complex interaction between microbes and the skin inflammation of atopic dermatitis (12). The majority of patients' non-lesion skin was dominated by coagulase-negative *staphylococcal* species (CoNS), particularly *S. epidermidis* and *S. hominis*. In accordance, other result (13) have shown that, when compared to non-lesion AD skin, acute and chronic lesion skin have lower relative abundances of CoNS and higher relative abundances of *S. aureus* (13). Even though the discovered CoNS frequently colonize moist skin, their abundances at the antecubital fossa were lower on healthy control skin than on AD skin without lesions. It could thus be hypothesized that the skin ecology in AD supports enhanced *staphylococcal* growth on both lesion and non-lesion skin and that changes in the distribution among the *staphylococcal* species towards greater abundances of *S. aureus* can contribute to the development of eczema locally on the skin. Another hypothesis, (14) shows that infants with atopic eczema and concomitant allergen sensitization present an altered gut microbiome and metabolome in the first 3 weeks of life(14). These early events were characterized by an enrichment of *Escherichia coli* and *Klebsiella pneumoniae* accompanied by increased gene expression of virulence factors (invasion, adhesin, flagellin and lipopolysaccharides). However, *S.*

epidermidis was the predominant isolate from the healthy area (57.34%) of AD patients, and *E. coli* was more prevalent in the healthy area than the lesion area. On the other side, each of *Klebsiella pneumoniae, Acinetobacter, Proteus sp.*, and *pseudomonas* percentages were higher in the lesion area than the healthy area. Likewise, researcher in Iraq (15) found that only 54 samples (36%) of *Staphylococcus aureus* isolates were given the typical biochemical tests and characteristics of morphology (15).

Table (4): Distribution of Bacterial isolates in cases from Atopy lesion and cases from healthy area

Bacterial isolates	Total no. of patients' 100					Total no. of healthy people 100	(%)
	No. of cases from 100 Atopy lesion	(%)	No. of cases from 100 healthy area	(%)			
S. aureus	80	80.00	40	40.00	0.0003 **	0	0.00
S. epidermidis	50	50.00	70	70.00	0.0679 NS	30	30.00
S. lentus	30	30.00	35	35.00	0.535 NS	0	0.00
P. aeruginosa	40	40.00	15	15.00	0.0007 **	0	0.00
E., coli	20	20.00	50	50.00	0.0003 **	5	5.00
Proteus Spp.	9	9.00	10	10.00	0.818 NS	1	1.00
K., pneumonia	35	35.00	3	3.00	0.0001 **	1	1.00
A., baumaani	15	15.00	8	8.00	0.144 NS	1	1.00
P-value	--	0.0001 **	--	0.0001 **	---	--	0.0001 **

** (P≤0.01), NS: Non-Significant.

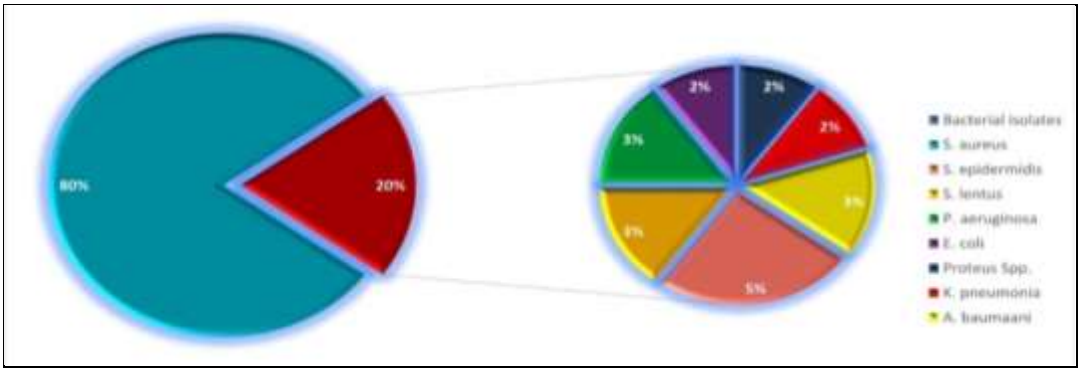

Figure (2): Percentage of bacterial isolates from atopic dermatitis lesions.

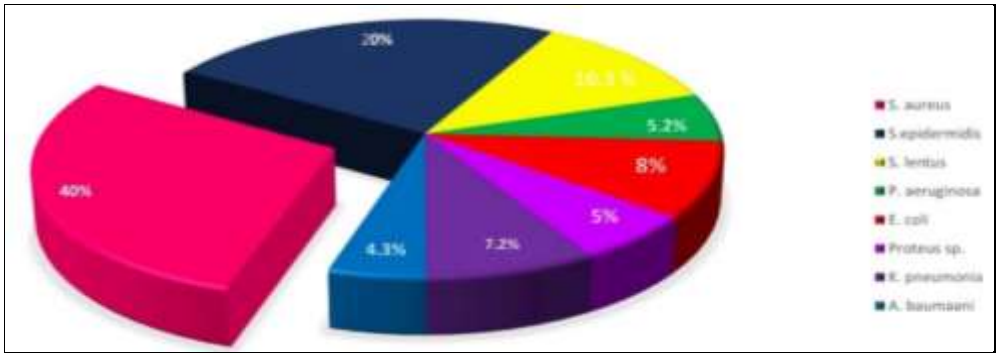

Figure (3): Percentage of bacterial isolates from atopic dermatitis nearly healthy area.

The AST was conducted on all *S. aureus* isolates from atopic dermatitis lesions showing a variable level of resistance to 20 antibiotics (Figures 4,5). Isolates showed a higher resistance levels to B-lactam group including (Ampicillin 9.1%, piperacillin 9.1% , Oxacillin 8.5% and Amoxicillin 7.8%) and 1st generation Cephalosporin group (Cefalexin 9.1%) followed by Macrolides group including (Azithromycin 8.3%, Clarithromycin 8.3% and Erythromycin 7.1%), the moderate resistant against Tetracycline group include (Tetracycline 7.2%, Minocycline 4.8% and Doxycycline 3.5%) and Lincosam group (Clindamycin 7.6%), while the higher sensitivity against Oxazolidinones group (Linzide 8.1%) and Glycopeptides group (Vancomycin 8.1%) followed by Fluroquinolone group include (Norofloxacin 8.1%, Ciprofloxacin 6.2% and Moxifloxacin (6.2% and Aminoglycoside group (Gentamycin 6.2%). This result is closest to the result in Iraq (16), showing higher susceptibility of *S. aureus* against the B-lactam group and the Cephalosporins of the 3rd generation class and not an equal result against Tetracycline group (high resistance) and Fluroquinolone group (moderate resistance). Other results in Iraq (17) agreed with the present result showing higher resistance of *S. aureus* against Penicillin and moderate resistance against Tetracycline and Macrolides groups. Therefore, according to the researchers (8) in Iraq, 14 isolates of *S. aureus* determine their sensitivity towards Amikacin, Impenem, Ciprofloxacin and Gentamicin (8). On the other hand, susceptibility against Aztreonam, Ceftriaxone, Amoxicillin, Penicillin, Cefixime, Ceftazidime and Metronidazole. Another Iraqi researcher (18) presents the high susceptibility of *S. aureus* against Amoxicillin, Ampicillin and Augmentin and high sensitivity against Vancomycine, Ciprofloxacin and Amikacin (18).

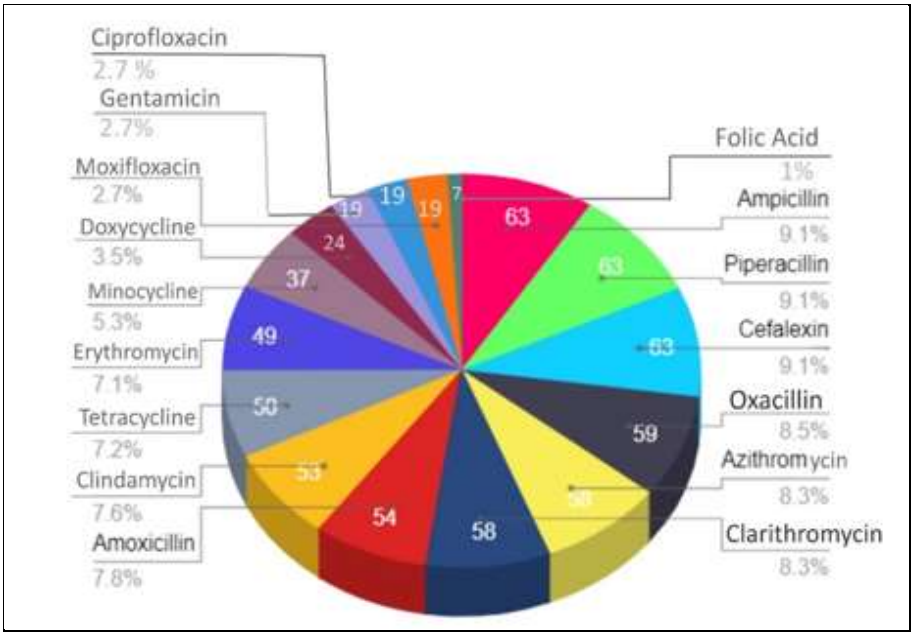

Figure (4): The susceptibility of *S. aureus* isolates

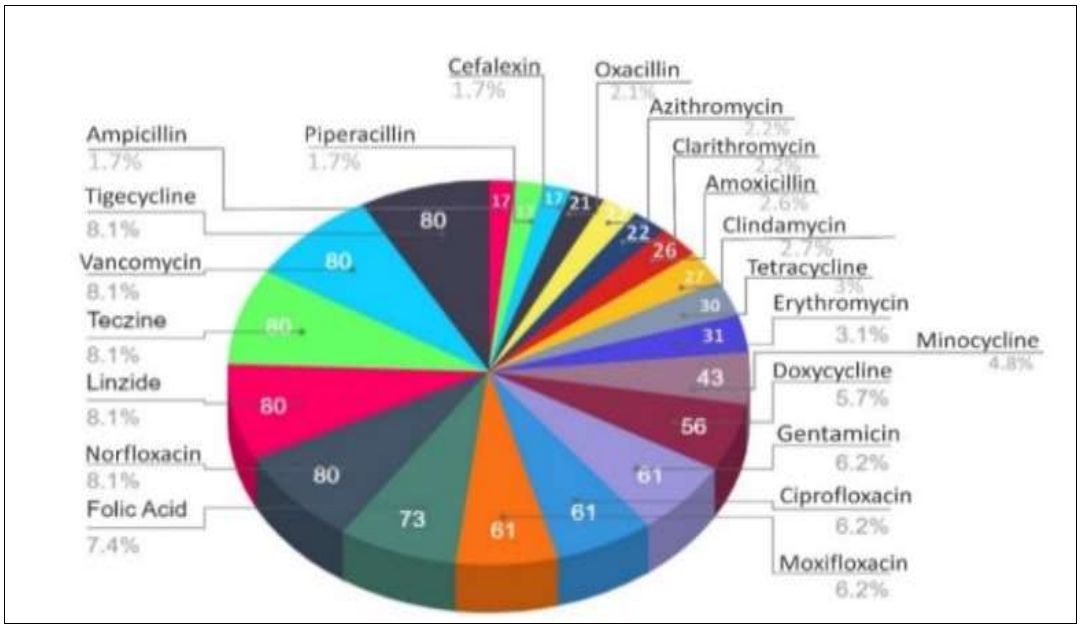

Figure (5): The sensitivity of *S. aureus* isolates

After that, molecular identification for *S. aureus* species was performed in the lesion area by using PCR for *16SrRNA* and *SPA* genes to confirm *S. aureus* identification. First, DNA was extracted from all isolates taken from lesion area, 80\100 (80%) samples that were phenotypically characterized as positive *S. aureus* isolates; concentration and purity were measured between 23 and 29 ng/l. The PCR has been conducted using sets of primers newly designed for this study. The results showed that 80/100 (80%) bacterial isolates belong to *S. aureus*, while the result for the *SPA* gene was 58 (72.5%) from the 80% *S. aureus* isolates carried *SPA* gene , as shown in (Figures 6 , 7). Researchers in Iraq diagnosed 21 isolates from 63 isolates as *S. aureus* using *16SrRNA* from different tissue samples, which is not identical with the current result (19). Such study found 41 isolates (50.6%) were identified as coagulase-positive *Staphylococcus aureus* from 81 samples, which did not agree with the current result, while the *SPA* gene could be detected in MRSA isolates by conventional PCR with a percentage of 83.3%, which is the most similar to the current result (7). Meanwhile, they found that the frequency of strains with short *SPA* bands in those isolated from patients was much higher than that of those isolated from healthy carriers. This led to the conclusion that the length of the spa gene depended either on methicillin resistance or the source of *S. aureus* isolation (20). Other result (21), found that in over half (62%) of the tested isolates, the presence of the *SPA* gene was detected in a given isolate. Those researchers concluded that mutations in the primer binding region might have missed spa-positive strains. This indicates that the strains were still virulent and invasive in spite of being spa-deficient. Also, the researchers (22) found evidence that some hospital patients have spontaneous spa-gene deletions, indicating that antibiotic pressure may be a factor in the genetic rearrangements of the *S. aureus* protein A gene.

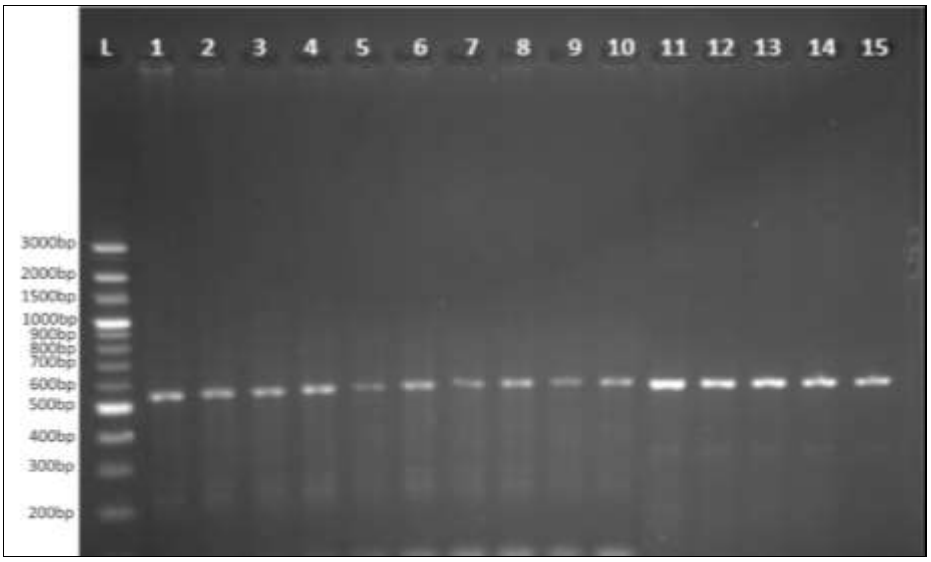

Figure (6): Agarose Gel Electrophoresis of PCR Amplified Products for *16SrRNA g*ene. Lane (L): l00bp ladder, lanes (1-15): DNA with positive result with expected size 550 bp (2% Agarose, 80 V for 70 min).

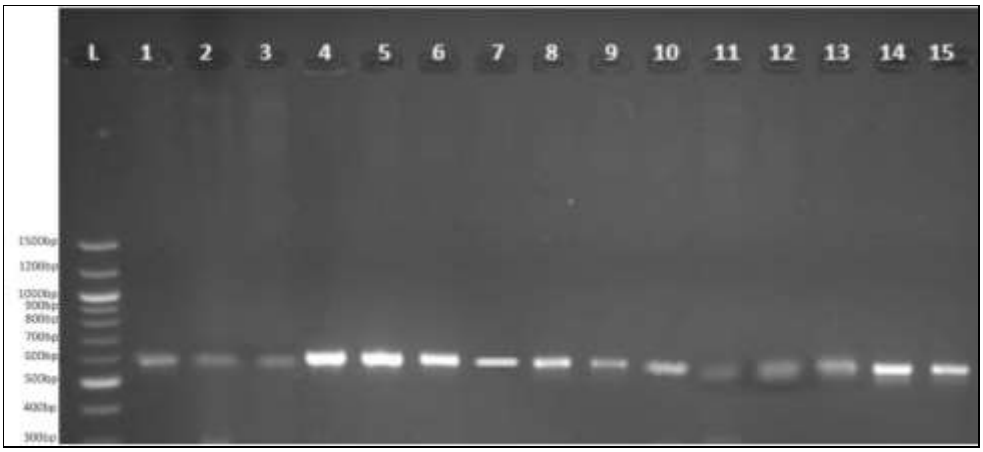

Figure (7): Agarose Gel Electrophoresis of PCR Amplified Products for *SPA* Gene. Lane (L): l00bp ladder, lanes (1-15): DNA with positive result with expected size 614 bp (2% Agarose, 80 V for 70 min).

Conclusion

Gram-positive bacteria, accompanied by other gram-negative specifically *S. aureus*, appeared to be the most common bacterial agent that caused secondary bacterial infection with Eczema, which considered multidrug resistance with high resistance to B-lactam groups and higher sensitivity against Oxazolidinone groups.

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