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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* AND *ACINETOBACTER BAUMANNII* ISOLATED FROM BABYLON HOSPITALS

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Abstract

Increasing resistance to antimicrobial agents used in the treatment infections with *S. aureus* and *A. baumannii* strains has become an important concern. Isolation and identification of *S. aureus* and *A. baumannii* strains from patients of different sources and detection of their antimicrobial susceptibility. Antibiotics resistance of 38 *S. aureus* and *A. baumannii* strains isolated from the samples. Identification of *S. aureus* and *A. baumannii* isolates were determined by mannitol salt agar and CHROMagar Acinetobacter¸ biochemical tests and automated system VITEK 2. Antimicrobial susceptibility tests were performed by agar disk diffusion and broth microdilution methods. Showed high resistance of *S.aureus* and *A. baumannii* to many antibiotics such as: Gentamycin, Trimethoprim / sulfamethoxazole, Ciprofloxacin and Levofloxacin (76%, 64%, 68%, 56%) for *S. aureus* (84.61%, 61.53%, 69.23%, 69.23%) for *A. baumannii*, respectively. while other isolates revealed low resistance for antibiotics such as: Doxycycline and Tetracycline (12%, 36%) for *S.aureus* and (15.38%, 15.38%) for *A. baumannii*, respectively. Whereas, methicillin resistance rate was (88%) by *S.aureus* bacteria.

Key words : *Staphylococcus aureus*, *Acinetobacter baumannii*, Antimicrobial susceptibility.

Introduction

Staphylococcus auerus and *Acinetobacter baumannii* of the most important pathogens that cause outbreaks in hospitals and serious health care associated complications in hospitalized patients, *S.aureus* and *A. baumannii* has become endemic in hospitals due to their versatile genetic machinery, which allows it to quickly evolve resistance factors, and to its remarkable ability (Corbella *et al.,* 2000 ; den Reijer *et al.,* 2018). After the revolutionary discovery of antibiotics, the medical community thought that the battle against microorganisms was won (Reffuveille *et al.,* 2017). However, the prevalence of drug-resistant bacterial pathogens is beginning to pose a major threat to global health due to misuse of antibiotics (Harkins *et al.,* 2017). The most alarming problems encountered are the ability of this species to have different mechanisms of resistance and the emergence of strains that are resistant to all commercially available antibiotics coupled with the lack of new antimicrobial agents, this has resulted in a limited choice of antibiotics for treatment of multidrug resistant isolates of *A. baumannii* (Foster., 2017; Lolans *et al.,* 2006 ; Urban *et al.,* 2003). There were increasing number of hospital outbreaks caused by *A. baumannii* has been reported from several countries around the world. In addition, inter-hospital spread of multidrug resistant *A. baumannii* has been observed as well as spread among countries (Visca *et al.,* 2011). This study was designed to isolation and identification of *S.aureus* and *A.baumannii* from different infections of hospitalized patients in Babylon hospitals, also this work aims to investigate antimicrobial susceptibility patterns among different *S.aureus* and *A.baumannii* isolates.

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Materials and Methods

Bacterial isolates

A total of 38 *S.aureus* and *A.baumannii* isolates were collected from 73 clinical specimens from different sources of patients at Babylon hospitals, Iraq during Beginning of March/2019 finished in the end of April 2019.

Bacterial isolation and identification were performed using standard laboratory methods. The isolates were non-repetitive, meaning that each isolate was obtained from a particular patient and each patient was sampled only once. Samples were streaked across mannitol salt agar and CHROMagar Acinetobacter (CHROMagar, France), MacConkey and blood agar plates for all specimens. Presumptive identification was done based on culture characteristics, gram stain, conventional biochemical tests and Vitek 2 system (Harley., 2016).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by agar disk diffusion, according to manufacturer instructions and Clinical and Laboratory Standards Institute (CLSI., 2019) guidelines. The applied antimicrobials were as follows : quinolones class (levofloxacin 5µg), aminoglycosides class (Gentamaicin 10µg), folate pathway inhibitors class (Trimethoprim\ sulphamethoazole 1.25/23.75mg), Tetracycline class (Doxycycline 30µg), Tetracyclines class (Tetracycline 30µg), fluoroquinolone class (Ciprofloxacin 5µg) and Penicillin class (methicillin 5µg) was checked against *S.aureus* bacteria only.

The growth inhibition zones around each disk were measured. broth microdilution method Mueller-Hinton broth was used according to the CLSI guidelines. MDR *S.aureus* and *A.baumannii* was defined as an intermediately-resistant or resistant isolate to more than two of the antimicrobial agents

Results and Discussion

Distribution of bacteria According to Infection

'The results revealed that a total number of 38(52%) isolates distributed of (25) *S.aureus* and (13) *A.baumannii* were obtained from 73 clinical specimens which had been initially diagnosed in hospitals from various body sites of infections. The distributions of the isolates according to specimens types are shown in table 1.

The percentage of *S.aureus* and *A.baumannii* isolates showed that there were highly significant differences to LSD values at ($p<0.01$) according to sources, and as follows: It found that the isolates were isolated in a high percentage swab of wound infection 8(32%) *S.aureus* and 4(30.76%) *A.baumannii*, swab of burn infection 7(28%) *S.aureus*, and 6(46.15%) *A.baumannii*, specimens from blood 5(20%) S.aureus and 2(15.38%) *A.baumannii*, specimens from urinary tract infection 3(12%) *S.aureus* and 1(7.69%) *A.baumannii*, from sputum of respiratory tract Infection 1(4%) *S.aureus* and 0 *A.baumannii*, from ear swab 1(4%) *S.aureus* and 0 *A.baumannii*. respectively.

This results was symmetrical to Aqeeli (2019) and Alagely (2016) as they reported their isolation of *S.aureus* bacteria from sources similar to the current study which is (wound, burning. Urine. Blood, Sputume).

Also agreed with the local studies in Iraq conducted by Al-Harmoosh (2015), Sahar and AL-Yasseen (2014) They diagnosed and identified that isolations in different percentage rates of nosocomial infections caused by *A.baumannii* obtained from the same sources and that agreement with present study.

Culturing Examination

In the present study, all 38 isolates were examined primarily for colony characterization after culturing on

Type of infection (specimens)	No. of samples	Percentage (%)	No. of *S.aureus* isolates	Percentage (%)	No. of *A.baumannii* isolates	Percentage (%)	Other types	Percentage (%)
Burn Infection(Swab)	22	(30.13%)	7	28%	6	46.15%	9	25.71 %
Wound Infection(Swab)	19	(26.02%)	8	32%	4	30.76%	7	20 %
Specimens (Blood)	14	(19.17%)	5	20%	2	15.38%	7	20 %
Specimens (Urine)	12	(16.43%)	3	12%	1	7.69	8	22.85 %
Respiratory Tract Infection (Sputume)	4	(5.47%)	1	4%	-	-	3	8.57 %
(Ear swab)	2	(2.73%)	1	4%	-	-	1	2.85 %
Total	73	100%	25	100%	13	100%	35	100 %
Chi-Square (x^2) (P-value)	32.872** 0.0003							

** P<0.01

** highly significant P<0.01

the selective media Mannitol Salt Agar (typical staphylococcus medium), CHROMagar (typical Acinetobacter medium), MacConkey agar and 5% ml human blood agar. They were then incubated for 24 hrs at 37ºC. The appeared colonies of *S.aureus* on Mannitol Salt Agar shape golden-yellow along the lines of streak reflecting mannitol fermentation. This indicate the positivity of this test which is a unique feature of *S.aureus* (Leboffe and Pierce, 2010). While, *A.baumannii* isolates on CHROMagar appeared as bright salmon-red colonies with special characteristic (Moran-Gilad *et al.,* 2014). as shown in Fig. 4-3.

The presence of this different color guide referred to the degradation of the artificial chromogenic substrates (chromogens) contained in the media by bacterial enzymes during the metabolism resulting in unique coloration of the colonies for each bacterium. These substrates enabling color-based identification of colonies recovered within 18 to 24 hours of inoculation. The degraded chromogens allowed easy identification of mixed growth and provided higher detection rates (Ajao *et al.,* 2011; Manickam *et al.,* 2013).

Biochemical Identification

A series of biochemical tests were performed to

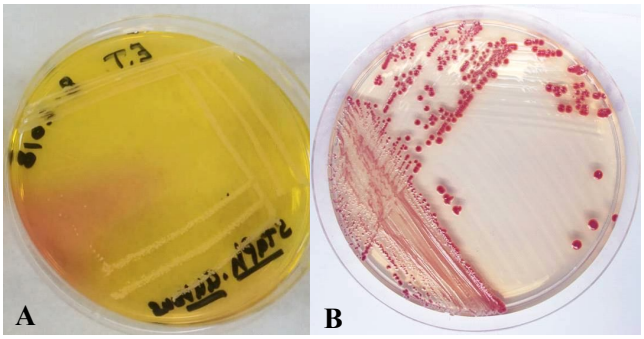

Fig. 1: (A)Colonies of S. aureus growth on mannitol salt agar. (B) Colonies of *A.baumannii* on CHROMagar *Acinetobacter.*

identify the bacteria listed in table 2. These tests allowed quickly to identify the unknown isolate based on the color changes that occur in the various tested. *S.aureus* and *A.baumannii* isolates gave in biochemical tests after 24 hrs of being incubated at 37°C. In case of catalase test, all the suspected isolates showed positive results for this test by the formation of gas bubbles after adding a hydrogen peroxides reagent to colonies. Howevers they gave negative results to the oxidase test. They gave alkaline acid type of growth slant and did not change the bottom and H_2S negative without gas production due to the fact that they it was strictly aerobic.

Biochemical tests showed that all isolates negative on indole production, motility test gave negative results., While the urease test gave negative results for *S.aureus*. But it gave variable results to *A.baumannii*. and *S.aureus* isolates gave a positive result for the lactose and coagulase test, while the result was negative in *A.baumannii* isolates.

In the present study, these conventional biochemical tests that carried out, and the results were compared with the standard result documented by Macfaddin, (2000) and Lahiri *et al*., (2004).

Identification bacteria species by *VITEK-2* System

This system has been used in many preceding studies because it gave good results for the identification and

Table 2: Morphological and Biochemical Identification Results of *S.aureus* and *A.baumannii.*

No.	Biochemical Test	Result	
		S.aureus	***A.baumannii***
1	Gram stain	+	-
2	Microscopic shape	cocci	Coccobacilli
4	Lactose fermentation	+	-
5	Oxidase test	-	-
6	Catalase production	+	+
7	Coagulase	+	-
8	Urease production	+	Variable
9	Motility test	-	-
10	Indole production	-	-

(+): positive; (-): negative.

confirmation of the biochemical tests. All 38 clinical isolates were identified as *S.aureus* and *A.baumannii* using Vitek-2 compact. The latter is the standard biochemical identification system for bacteria and is used to confirm the identification of bacterial isolates. This apparatus showed strong results in the reaction test. The BioMerieux Company that synthesized this apparatus remarked that the accuracy of this system (GN Card) for Gram negative strains. The analytical profile index of this system showed 99.99% identification percentage probability. Garrote *et al*., (2000) mentioned that Vitek-2 system is ables to accurately give a rapid identification of clinically significant bacteria.

4.5.1 Antibiotic Susceptibility Test

The results revealed that often *S.aureus* and *A.baumannii* clinical isolates have a very high level of resistance to the antibiotics under test as presented in Figs (2, 3) which represent the antibiogram profile of the isolates. They further indicate that isolates which vary in their susceptibility to the antibiotics.

Many previous studies revealed that *S.aureus* is one

of the most common gram positive bacteria that can cause nosocomial infection in health care centers and burn units

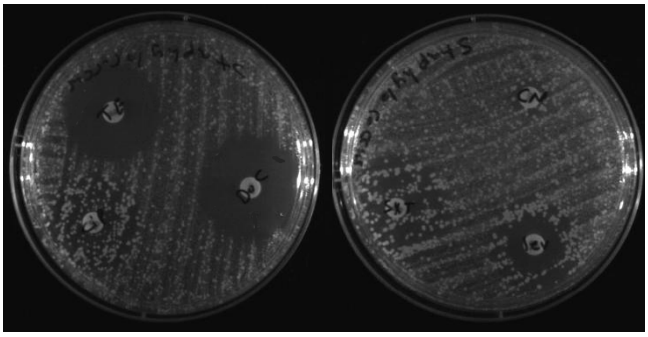

Fig. 2: Antibiotic Susceptibility Test for *S.aureus* and *A.baumannii* Using 6 Antibiotics.

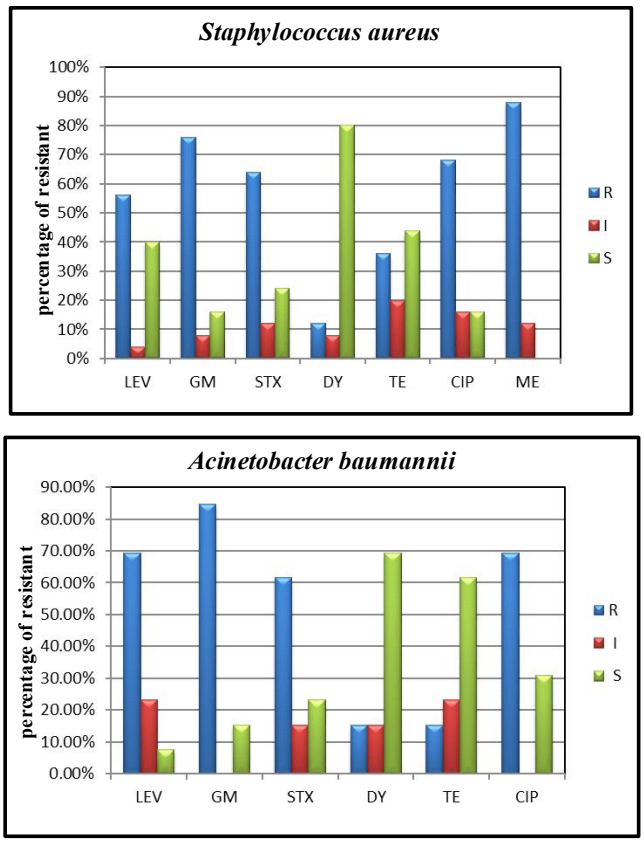

Fig. 3: The Percentage of Susceptibility (Resistance, Intermediate and Sensitive) for *S.aureus* and *A.baumannii* Isolates against Antibiotics.

in burned hospitalized patients (Gitau *et al.,* 2018). Multidrug resistant *S.aureus* is the most common cause of nosocomial infection in burn patients in teaching medical Al- Kendi hospital (Alwan *et al.,* 2011).

The results demonstrated that out of (25) tested *S.aureus* isolates, (22) isolate (88%) were resistant to methicillin (MRSA) while the rest (3) isolate (12%) were intermediate. (methicillin is a narrow-spectrum β-lactam antibiotic of the penicillin class) methicillin acts by inhibiting the synthesis of bacterial cell walls (Cui *et al.,* 2006). Methicillin is actually a penicillinase-resistant β -lactam antibiotic. Penicillinase is a bacterial enzyme produced by bacteria resistant to other β- lactam antibiotics which hydrolyses the antibiotic, rendering it nonfunctional. This result was close with Aqeeli (2019) found that among 96 *S.aureus* isolates tested, 91 isolates (94.79%) were methicillin-resistant while the rest (5 isolates) (5.21%) were intermediate.

The results of the current study in Fig. 3 showed how sensitive *S.aureus* and *A. baumannii* were in 6 antibiotics using the Kirby-Bauer tablet diffusion method, and growth in Mueller-Hinton agar (MHA) showed high resistance of *S.aureus* and *A.baumannii* to many antibiotics such as: Gentamycin, Trimethoprim / sulfamethoxazole, Ciprofloxacin and Levofloxacin (76%, 64%, 68%, 56%) for *S.aureus* (84.61%, 61.53%, 69.23%, 69.23%) for *A.baumannii*, respectively. while other isolates revealed low resistance for antibiotics such as: Doxycycline and Tetracycline (12%, 36%) for *S.aureus* and (15.38%, 15.38%) for A.baumannii, respectively. Whereas, methicillin resistance rate was (88%) by *S.aureus* bacteria.

These results were similar and quite agreement to the pervious studied in Iraq by AL-Kadmy *et al*., (2018); AL-mousawi, (2018) who reported that the resistance rate of *A.baumannii* isolates to levofloxacin, gentamicin, trimethoprim / sulfamethoxazole, ciprofloxacin, cefepime and ceftazidime was high Up to 90%.

Results of this study obtained similar with results (Saba *et al*., 2017) that the MRSA isolates were resistant to trimethoprim-sulfamethoxazole as 63.54% percentage. And agreed with a study in Iran, Shokouhi *et al*., (2017) reported that 20% of *S.aureus* were resistant to doxycycline.

Results conducted in current study agreement with Genteluci *et al*., (2016) who reported that *A.baumannii* isolates developed resistance to different antibiotic classes, including flouroquinolones in high resistance rates. The resistance to flouroquinolones, including Ciprofloxacin, was due to mutations in some of these bacteria, resulting in a change in the membrane permeability (Lahiri *et al.,* 2004).

With regard to fluoroquinolone resistance of *A. baumannii,* it is often caused by modifications in the structure of DNA gyrase or topoisomerase IV secondary to mutations in the quinolone resistance-determining regions of the *gyrA* and *parC* genes. It is the main cause for resistance of *A.baumannii* to quinolones. These changes result in a lower affinity for the binding of the

quinolone to the enzyme-DNA complex. A second mechanism of resistance to the quinolones is mediated by efflux systems that decrease the intracellular drug accumulation (Potron *et al.,* 2015).

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