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The identification of *Serratia* spp. isolated from urinary tract infection in Mosul city, Iraq

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Abstract
Serratia fonticola and *Serratia odorifera* are highly multi drug resistance like other opportunistic pathogen associated with nosocomial infection, we were collected 200 urine samples all of them cultured on selective media, all isolates were highly resistant100% to beta-lactam antibiotics Cephalothin, Ceftriaxone, Amoxicillin, Ceftazidime and fluoroquinolone Norfloxacin and Ciprofloxacin, the resistance for, Aztreonam, Levofloxacin, were 50% and Nitrofurantoin 25% The concentration of Minimum Inhibitory test for Nitrofurantoin is 50 µg/ml and for Levofloxacin is 100 µg/ml. Depending on identification by automated system (VITEK2) we obtained respectively *Serratia fonticola* and *Serratia odorifera* (94% and 89%). The molecular detection of *gyrB* gene showed that 75% of isolates were owen this gene at 910bp.

Keywords: *Serratia fonticola*, *Serratia odorifera*, Vitek2, *gyrB*

Introduction
These to species are G^- bacteria from Enterobacteriaceae group as opportunistic pathogens and caused disease as nosocomial infections some of them difficult to treatment because contain multi-drug resistance, these bacteria responsible to causing many clinical diseases, from different systematic organs in human like urinary tract infections UTIs [1].
S. fonticola has special biochemical specification differ from other *Serratia* species, like their behavior to gelatin hydrolysis or production of DNase it has a % G+C (49 to 52%) whereas other *Serratia* spp. has (52 to 60%) despite of theses difference they are until found in *Serratia* species. Depending on 16s rRNA analysis [2].
S. odorifera do not contain pigments or produce them, it named depending on odor produced from them like potato, scientists like Gavini and others found that twenty microorganisms had same characteristics isolated from waters.
In 1988, 1st case recorded in adult infection caused by *S. odorifera* in male (67) with cirrhosis and septic shock with this bacteria under bio type one was isolated from each blood and urine [2].
UTIs bacteria were colonized urethra and blushed out during urination, females more than male because the physiological and anatomical urogenital tract differ from men, makes bacteria colonize this area [3].
UTI includes (pyelonephritis, fetal mortality especially in pregnant women and disorders in renal function in infants and kids), doctors recognizable these diseases depending on clinical symptoms and lab tests. [4, 5].
The resistance to ciprofloxacin antibiotic that belongs to fluoroquinolones group it associated with DNA gyrase and topoisomerase IV the changes happen in topoisomerase enzyme led to reduce its affinity to fluoroquinolones [6].
Serratia spp. resistance *gyrA* and *gyrB* depending on mutational alterations in the target genes [7].
The aims of our study are to isolate and identified these rarely bacteria from UTI patients in Mosul city traditionally and by VITEK2 and molecularly, then detect their sensitivity towards antibiotics to detect the drug of choice for these bacteria.

Material and Methods
Bacterial strains: we were collected 200 isolates from UTI patients coming in Al-Salam general teaching hospital and Ibin-Seena hospital in Mosul city, IRAQ from 1/ October /2020 to 1/ February / 2021, all samples collected in Caprylate thallous (CT) agar as selective media for isolation *Serratia* spp. from clinical samples, this media composite by three parts Trace elements solution H_3PO_4 1.96 g, $FeSO_4 \cdot 7H_2O$ 0.0556 g, $ZnSO_4 \cdot 4H_2O$ 0.0287 g, $MnSO_4 \cdot 4H_2O$ 0.0223g, $CuSO_4 \cdot 5H_2O$ 0.025 g, $Co(NO_3)_2 \cdot 6H_2O$ 0.003 g, H_3BO_3 0.0062 g and D.W. 1000 ml, sol. A $CaCl_2 \cdot 2H_2O$ 0.0147 g, $MgSO_4 \cdot 7H_2O$ 0.123 g, KH_2PO_4 0.680 g, K_2HPO_4 2.610 g, Trace element solution (see above) 10 ml, Caprylic acid 1.1 ml, Yeast extract (5% wt/vol solution) 2 ml, Thallous sulfate 0.25 g, Distilled water up to 500 ml and adjusted pH to 7.2 and sol.

B NaCl 7 g, (NH4)2SO4 1 g, Agar 15 g and D. W. 500 ml [8]. Then transport the isolates to Luria -Bertani Agar (LB agar) Incubated at 37c in 24h.

Biochemical Identifications

A. Traditional methods

Depending on Bailey and Scotte we achieved several Biochemical tests for all suspected bacteria under study, these tests included gram stain, catalase, oxidase, IMViC, Lactose fermentation, and Triple Sugar Iron test, IMViC is important because we can differentiate between the Enterobacteriaceae family [9].

B. Detection of *Serratia* spp, depending on Regnum Prokaryote

We used this online software for detection gram positive and gram-negative bacteria depending on biochemical results http://www.tgw1916.net/index.html [10].

C. Automated method

All isolates underwent to identify with VITEK-2 system that has high accuracy to the detection of gram-negative bacteria like *Serratia* spp. the test achieved by manufactured instruction.

Anti-bio gram Test

According to Sharma [11] and ours modification; the antimicrobial test were accomplished against bacteria under study due to Kirby-Bauer disc diffusion methods according to CLSI procedures, antibiotics used belongs to Bioanalyzer Company Amoxicillin/Clavulanic acid (AMC25µg), Aztreonam (ATM30µg), Cefalothin(30µg), Cefotazidime (CAZ30µg), Ceftriaxone (CRO10µg), Ciprofloxacin (CIP10µg), Levofloxacin (LEV5µg) Norfloxacin (30 µg), Ampicillin (30 µg), and Nitrofurantoin (100 µg) depending on McFarland 5 we were suspend bacteria and inoculation with aseptic swabs on Muller- Hinton agar, incubated at 37 ˚C for 24hrs.measured inhibited diameter [12, 13, 14].

Extract DNA

Extractions DNA for all bacteria under study done by Genomic DNA extraction kit (Geneaide, USA) followed the instruction of the company. Bacteria were grown in nutrient agar incubated at 37℃ for 24h.then a full loop inoculation were taken to performed the extraction according to instruction of manufacturing company. Extracted DNA purity and concentration was measured by Biodrop, BD 1043 and reserve at -20 ˚C until PCR was performed [15].

PCR and primer

(PCR) mixture prepared depending on Master Mix instructions "GoTaq" Green promega, USA, it was composed from 10µl GoTaq Green Master Mix, 4µl Nuclease Free Water, 1µl F *gyrB* P: 5-TAARTTYGAYGAYAACTCYTAYAAAGT-3, 1µl R *gyrB* P: 5-CMCCYTCCACCARGTAMAGTTC-3 nd 4µl DNA under study. The technique was carried out using eppendrof thermal cycler model: Eppendrof AG, 22331, Hamburg, GERMANY [16].

Detection of *gyrB* (910 bp.) gene

The byproduct was observed with UV trans-illuminator (model MUV21-312, TAIWAN) after electrophoresed by agarose gel (2%) for 100 volts at 75minute, the gel was photographed using iPhone 8 plus digital camera, Table 1 [16].

Table 1: program of *gyrB*

Initial Denaturation		35 Cycles						Final Extension		Cooling	
		Denaturation		Annealing		Extension					
Temperature	T	Temperature	T	Temperature	T	Temperature	T	Temperature	T	Temperature	T
94	4 minutes	94	1 minute	55	1 minute	72	2 minutes	72	10 minutes	4	3 minutes

Results and Discussion

Two hundred samples of urine were collected from Ibin -Sena teaching hospital and ALMADINA clinical laboratory from October/2020 to January/2021 in Mosul city, Iraq. all samples underwent to several tests for identifying the bacteria by traditional and automated methods.

Depending on biochemical tests we obtained *Escherichia* spp.) n=45; 22.5%) *Klebsiella* spp. (n=15; 7.5%), *Enterobacter* spp. (n=3;1.5%), *Serratia* spp. (n=4 ;2%) samples belong to the genus *Serratia* spp., we were detecting only 4 isolates belong to these bacteria depending on VITEK2 automated system, these isolates return to *S. fonticola* and *S. odorifera* (Figure 1) and (Table 2).

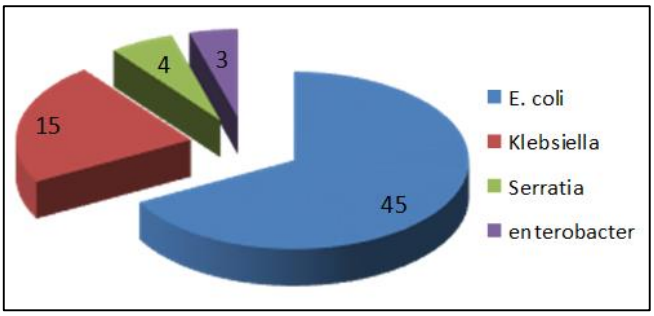

Fig1: The distribution of *Serratia* spp. among all other pathogens isolated from urine

Our results similar somewhat to Mantadakis, and collegues they obtained several bacteria from 218 UTI infections were belongs to *E. coli* (76.9%), *P.* spp. (7.7%), *K.* spp. (6.8%), *P. aeruginosa* (4.1%), *E. faecalis* (1.8%), *Enterobacter* spp. (2; 0.9%), *M. morganii* (0.9%) and *Serratia fonticola* (2; 0.9%) [17].

This study compatible with our study in the way of pathogens percentage occurrence for both *Serratia* and *Enterobacter* and alittile bite with *E.coli* and *Klebsiella spp.* others study on *Serratia* frequency in urinary infection agree with ours done by Menezes and coworkers [17, 16, 19, 20].

Dr.Kujur, made his study on pathogens causing catheter associated UTI and his isolate arranged as this percentage predominance for *E.coli*, (31%) followed by enterococcus spp. (29%), *Klebsiella* spp (17%), *Acinetobacter* spp (14%), *Citrobacter* (4%), *Serratia fonticola* (1%) [21].

Table 2: Biochemical test for both *Serratia fonticola* and *Serratai odorifera*

Biochemical tests	*Serratia fonticola*	*Serratia odorifera*
Gram stain	-	-
Catalase	+	+
Oxidase	-	-
I	-	-
MR	+	+
VP	-	-
C	+	+
H_2S production	-	-
Lactose	+	+
Sucrose	+	+
Pigmentation	-	-
Motility	+	+

Based on the biochemical results we have used them in REGNUM PROKARYOTAE for the purpose of confirming the diagnosis before we undergo isolations to VITEK 2 system (Figure 2 and 3), using this software, it is very important for highly advanced detection in the microbiology domain and benefit for microbiologist and researchers in the whole world.

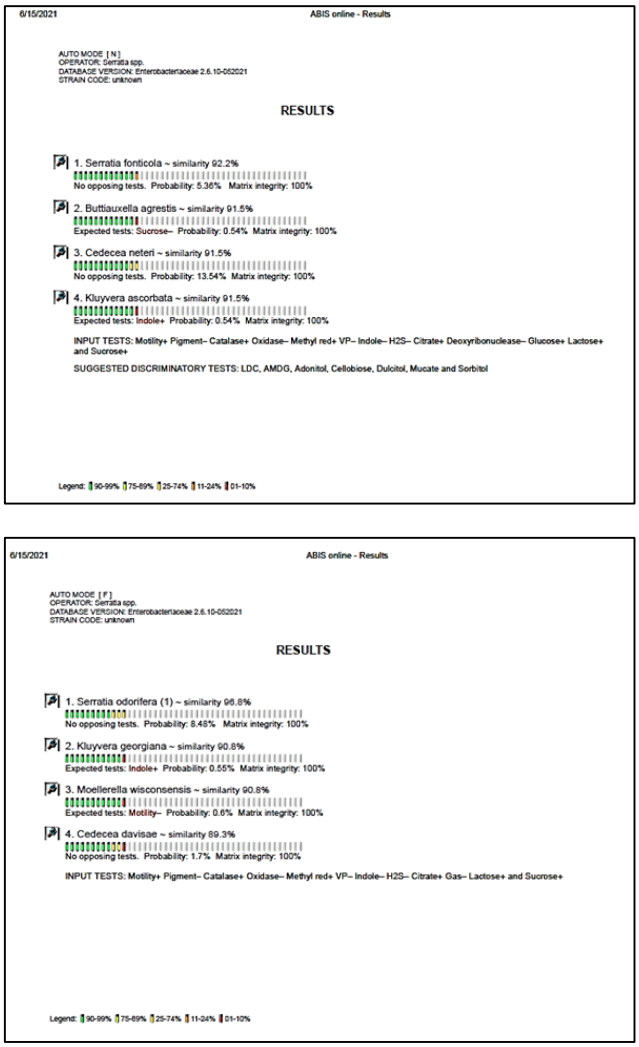

Fig 2, 3: The identification results of two isolates of *Serratia* spp. depending on REGNUM PROKARYOTAE.

Sulaiman pointed out that the use of this program is very important in bacterial diagnosis and of great benefit to researchers in the field of bacteria science as well as in antibiotic sensitivity tests [22].

Depending on identification by automated system (VITEK2) we obtained respectively *Serratia fonticola* and *Serratia odorifera* (94% and 89%), our results similar somewhat to Spanuu and Richter and their colleagues they identified bacteria with accuracy 96.7% he found that the accuracy of this test was 95.6% [23, 24].

The use of screening and manual tests alone is not sufficient in the clinical laboratory also it west the time and labor intensive there are several commercial automated systems like vitek 2 system used for identification bacteria with highly accurate and in short time, VITEK discovered at 1970s as automated system for bacterial diagnosis, antibiogram tests until now works with very good results for detection [25, 26].

In our study we found that these bacteria are multi drug resistance to several antibiotics Cephalothin, Ceftriaxone, Norfloxacin, Ampicillin, Amoxicillin, Ceftazidim and Ciprofloxacin in the rate of (100%), 50% for Aztereonam and Levofloxacin 25% for nitrofurantoin Table 3.

Table 3: The Antibiotic Sensitivity Test

Antibiotic, Abbreviation and Concentration(µg)	Results	R	I	S
Amoxicillin/ Clavulanic acid AMC_{30}	10.7	≤13	14-17	≥18
Cephalothin- KF_{30}	R	≤14	15-17	≥18
Cefitraxone- CRO_{10}	R	≤13	14-20	≥21
Aztreonam- ATM_{30}	12	≤15	16-21	≥22
Levofloxacin- LEV_5	9	≤16	17-20	≥21
Norfloxacin- NOR_{30}	R	≤12	13-16	≥17
Nitrofurantoin- F_{100}	17	≤14	15-16	≥17
Ampicillin- APX_{30}	R	≤11	12-13	≥14
Ceftazidim- CAZ_{30}	R	≤14	15-17	≥18
Ciprofloxacin- CIP_{10}	R	≤15	16-20	≥21

The antibiotics belongs to β-lactam are commonly used in worldwide. These group of antibiotics very useful against G^- and G^+ microbes, but some of these bacteria can hydrolyzing penicillin and cephalosporin's, then they resistant antibiotics, we need long term treatment options [27].

So, our study shows that all isolates were resistant to beta-lactam antibiotics in AL-najaf city they indicate that al isolates were resistant to 100% to ampicillin and amoxicillin; other study done by Şimşek [28] deal with *Serratia marcescence* resistant rates against ceftriaxone and ceftazidime respectively 22.7% and 19.6%, the clinical isolates of *Serratia* exhibited highest resistant to ceftriaxone, ceftazidime [26].

We absolutely agreement with the study of Gul, and Gurbuz that the resistance was 100% for Ampicillin, Ceftriaxone and Ciprofloxacin they also resistance to fluoroquinolones antibiotics is related to possessing the *gyrB* genes its product is gyras enzyme that interfere with bacterial DNA replication [19].

We detected *gyrB* gene with 910 bp was found in three isolates 75% of *Serratia* spp., this gene also uses to find the relationship many of *Serratia* species, and for detect their ability to resistance fluoroquinolones antibiotics Figure 4 [29, 30].

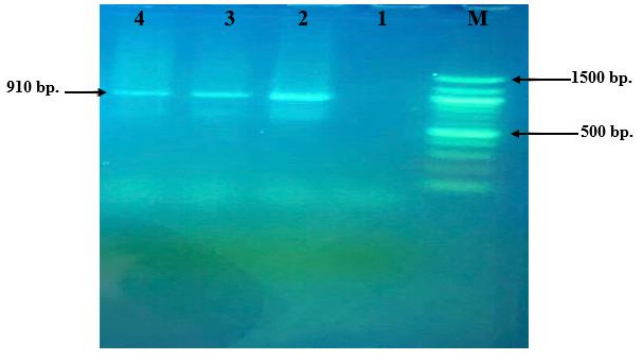

Fig 4: Electrophorasis of *gyrB* gene in both *Serratia fonticola* and *Serratia odorifera* M: ladder 1500 bp., 1, 2, 3 *S. fonticola*, 4 *S. odorifera*

Dauga found that the use of *gyrB* gene in determining the relationships among *serratia* spp is more reliable than use 16srDNA they detect that the similarity ranged between(98.9%)*S. piymuthica*, *S. liquefaciens*(94.2%) *S.rubidaea*(87.8%) and S. fonticola was (86.5%) [29].

Mutation in *gyrA* and *gyrB* lead to increase the flouroquenolons resistance rate this group of antibiotics act to inhibit the bacterial DNA replication via the inhibition of DNA gyrase and topoisomerase [31].

This gene encode the ATPase domain of DNA gyraseB-subunit protein as illustrated in the study of Ruan and colleagues, 2017 on 9 *Serratia* spp. to test specification of this primers and a number of other enterobacteriaceae family to test the cross- reaction of this primer they detect that this primer gyrB is specific for *S. fonticola* [32].

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