Molecular Profile of Integrase gene *intI* and Carbapenem gene in *Aeromonas sobria* Isolates

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Abstract

The current study includes the detection of *Aeromonas sobria* isolates were obtained from total 157 samples involved the clinical samples (diarrhea samples) from patients suffering from diarrheal infections during the period from April 2017 to November 2017. at AL-Central Health Laboratory in Najaf governorate. These isolates were diagnosed by four method as (Culture method, biochemical tests, Vitek@2GN cards system and Polymerase chain reaction (PCR) technique). TheVitek@2GN cards system was the best method for diagnosis, which have led to isolate and diagnosis of 33 isolate. Multidrug resistance (MDR) of bacteria were detected by MIC testing and performed with the automated VITEK@2 GN/AST compact system. A clear variation was observed in their susceptibility to 13 antibiotics disks, these isolates of *A.sobria* were revealed resistance for some antibiotics such as Penicillin class Methicillin, Amoxicillin, Ampicillin, Penicillin (100%), and (91.6%) for Carbenicillin, and high rates of resistance (100%) to Cephalosporins that represented by Ceftazidime, Cefotaxime, Cefoxitin and Cefepime. The investigation of mobile genetic elements among isolates demonstrated that*A.sobria* have been Class I integron genes (class 1 integron represented by integrase *intI1,* and IMP genes).

Keywords: *Aeromonas soria, IMP gene, integrase intI1, Antibiotics and imipenem.*

Introduction

Aeromonas sobria are species of the genus *Aeromonas*, which belongs to the family *Aeromonadaceae* that received increasing attention opportunistic pathogens because of its association with both diarrheal and extra intestinal infection in human disease especially in children and persons with impaired immune system[1,2,3]. Multidrug resistance (MDR) was reported in the genus of *Aeromonas*[4]. *A.hydrophila*and *A.sobria*are anantibiotic resistant a variety of antibiotics have been used to treat infection caused by *A.hydrophila* and *A.sobria*. The high occurrence of (MDR) observed in *Aeromonas spp.*[5,6]. The antibiotic resistance genes that integrons capture are located on gene cassettes. *Aeromonas spp*. have been contain Class I integrin[7]. Integrons may be found as part of mobile genetic elements such as plasmids and transposons. Integrons can also be found in chromosomes. The aim of studyfor this purpose, the steps were: 1- Isolation and identification of *A.sobria* in clinical and in Najaf by VITEK@ 2 GN/ID card system. 2- Detection the Antimicrobial susceptibility of *A.hydrophila* and *A.sobria* isolates using antibiotics disks and MIC/AST. 3- Characterization of Class I integron and related mobile genetic elements (MGE) among MDR isolates. And determination of some MDR and β-lactamase resistance genes such as (bla_{IMP}).

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Materials and Method

- **Identification of *Aeromona*:** Phenotypic properties were recorded on microscopic characteristics by

Gram's stain was used to examine the isolated bacteria for studying the microscopic characteristics such as gram reaction, shape, motile and the media that are used (TCBS agar; MacConkey agar and blood agar) for primary identification of *Aeromona*. Also Biochemical tests used Catalase test (3% Hydrogen Peroxide), Oxidase test, Indole Production test, Simmon's Citrate test and Motility test were all these tests and urease test result according to studies[8,9].

- **Identification of *Aeromonas* by VITEK@2 GN-ID System:** The identified *Aeromonas ssp* isolates were confirmed with the automated VITEK@2 compact system by using GN/ID cards. The GN ID card is based on established biochemical method (64 reaction) and newly developed substrates, measuring various metabolic activities.
- **Antibiotics susceptibility:** Minimum Inhibitory Concentration (MIC) antibiotics were used by VITEK@2/AST, such as (AST-GN084, AST-GN093 Card system), as mentioned in antibiotics disks were used 13 antibiotic of many class and sub-class antibiotics (Penicillins, Cephems, Monobactams, Nitrofurans, Quinolones, Ansamycin, β-lactamase, Carbapenem, Tetracycline, Aminoglycosides, Phenicols, Folate pathway inhibitors and Lipopeptide).

Results And Discussion

Isolation and Identification Culture and Biochemical Tests: The isolation and identification of *Aeromonas sobria* showed that only 33 isolate were positive based on the morphological characteristics of the colonies on TCBS, MacConkey agar, Aeromonas media and blood agar media These isolates were smooth yellow, shiny, flat, about 2-3 mm in diameter colonies on TCBS (Fig. 1), while they were small and pale colonies on MacConkey's agar when incubated for 24h. While microscopic examination of cultures showed that the bacteria were gram-negative slightly curved rods, non-spore forming cells, arranged as single or double of bacterium bacilli. The classification of *Aeromonas* has been confusing because of lack of matching between phenotypic and genotypic characteristics of species and multiple method that are required for accurate taxonomy[10].

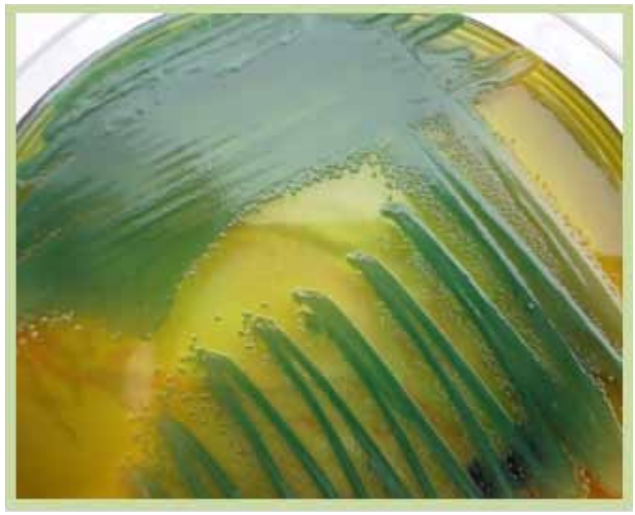

Fig. 1: *A.sobria* isolate on Aeromonas isolation agar and

On the other hand, the results of biochemical tests referred to that not all were positive to oxidase and catalase tests. The positive isolates were characterized with the ability to ferment the glucose only on KIA, so the isolates gave alkaline slant with acid bottom without H2S or CO^2 production. Also, isolates showed positive results to simmon's citrate and negative to urease test [Table 1]. According to these biochemical tests only 33 stool samples showed positive result as *A.sobria* . This result was predicted by previous studies[6,11,12].

Table 1: Biochemical tests for *Aeromonas*

No.	Tests	A.sobria
1	Oxidase test	+
2	Kligler iron agar	Alk/Acid with gas
3	Indole test	+
4	Citrate test	+
5	Methyl red test.	+
6	VP	+

The identification by VITEK@2 was performed with the automated VITEK@2 system using GN-ID cards which contained 64 biochemical tests. The results were demonstrated 33 *A.sobria* isolates were confirmed with ID message confidence level ranging excellent (Probability percentage from 94 to 99.7 %).

Molecular Identification by PCR Technique:

Antimicrobial Susceptibility Determination:

A. Minimum Inhibitory Concentration of Aeromonas: MIC testing was performed with the automated VITEK@2 GN/AST compact system. The results of the study revealed that all*A.sobria*isolates were resistant to minimum of many classes of antibiotics (MIC) to which they are tested. Hence, all isolates were considered to be multidrug resistant (MDR), revealed that the resistant of *A.sobria* isolates to Penicillins (Ampicillin, Amoxicillin/Clavulanic, Piperacillin and Piperacillin-Tazobactam) were recorded in 100% for all isolates . The resistance to β-lactam/β-lactamase inhibitor combinations was appeared in 100% of *Aeromonas* isolates. MDR of *A.sobria* isolates were represented by resistance to twelve class and sub class of antibiotic. All isolates appeared resistance to (Penicillin class) Methicillin, Amoxicillin, Ampicillin, Penicillin (100%), and (91.6%) for Carbenicillin. The study also revealed a high rates of resistance to Cephalosporins that represented by Ceftazidime, Cefotaxime, Cefoxitin and Cefepime were detected in (100%). Resistance to all β-lactam/β-lactamase inhibitor combinations includingAmoxicillin-Clavulanic acid and Pipracillin-Tazobactam (100%). The results of resistance isolates appeared high resistance to Imipenem (75%), Meropenem (77%) and Ertapenem (80%) of Carbapenem class, were effective against the majority of these isolates, these results agreed with other studies such as[4,13]**.** The human populations within these regions are at risk of exposure to antimicrobial resistant bacteria, and thereby disseminating antimicrobial resistance (ARGs) genes [14]**.**

Molecular Detection of Resistance and Integron Class I Genes: All *A.sobria* isolates were detected for the present of ESBL genes. The results revealed that isolates yielded amplification products with specific primers for types of extended-spectrum-β-lactamase(ESBL) and metallo-beta-lactamase (Carbapenem) antibiotics genes. All isolates in the present study were tested phenotypically of ESBL and Carbapenem production by MIC method and genotype. However, gene in the families only IMP were examined in the present study. Detection of these genes was performed by PCR technique. The results revealed that out of the 29 *A.sobria*isolates contained of genes 29 isolate *bla* $_{IMP}$(587 bp), *intI1*(497bp) *genes* as mentioned in [fig. 2] and [fig. 3]. Previous studies unquestionably established the role (>85% of isolates) of many *Aeromonas* spp. such as *A. hydrophila, A. Sobria, A.caviae*and *A. veronii*in diarrhea[4,15]. Deng *et al*[16] *confirmed that Aeromonas* strains containing multiple drug-resistance integrons, and these data suggests that surveillance for antimicrobial resistance of animal origin and responsible use of antimicrobials in aquaculture is necessary in these farms. Current results are agreed of *Aeromonas* produced class I integronsgenes with other studies[7,17,18].

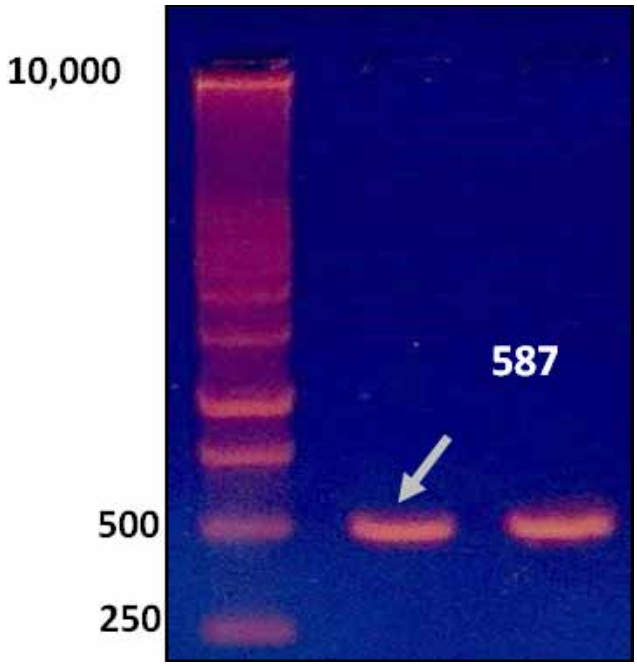

Fig. 3: Agarose Gel Electrophoresis (1.5%) of PCR products of *intI1*(497 bp) gene of .*sobria* isolates for (45) min at (100) volt.

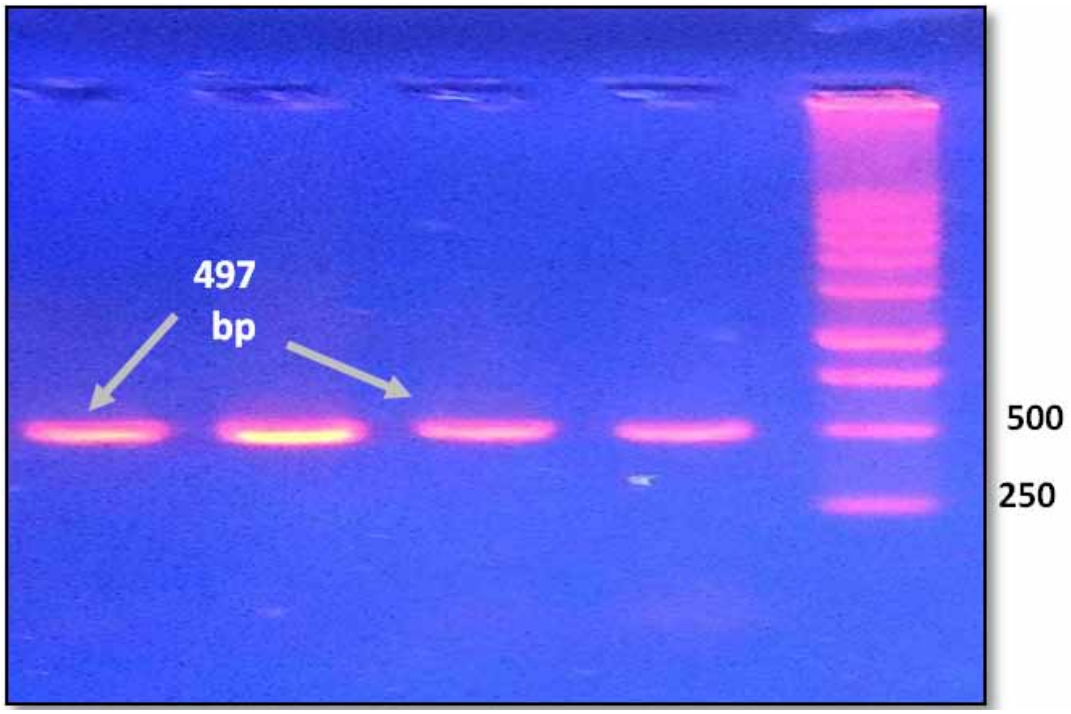

Fig. 2: Agarose Gel Electrophoresis (1.5%) of PCR products of IMP Genes (587bp) of *A.sobria* isolates for (45) min at (100) volt.

Conclusion

The frequency of *A.sobria* isolates in Najaf were higher among local clinical isolates. Identification by VITEK@2GN card system and Molecular techniques are necessary for detection of pathogenic bacteria among clinical isolates. Molecular characterization of Class I integrongenes, genetic elements (MGE) among MDR isolates were found in most *Aeromonas* isolates. *A.sobria*was multidrug resistance by shown a great emergence of MDR isolates among strains isolated and resistance to twelve class and sub class of antibiotic Methicillin, Amoxicillin, Ampicillin, Penicillin, Ceftazidime, Cefotaxime, Cefoxitin and Cefepime, Amoxicillin Clavulanic acid and Pipracillin-Tazobactam, Rifampin.

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