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DETECTION OF EXTENDED-SPECTRUM B-LACTAMASE (ESBLS) IN *AEROMONAS* AND *ACINETOBACTER SPP* ISOLATED FROM CLINICAL SPECIMENS

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ABSTRACT: *Acinetobacter and Aeromonas cause wide variety of infections in human including wound , burn ,urinary tract infections and diarrhea. The clinical specimens including (burn swabs, wound swabs, urine samples and stool samples) were collected between November 2015 and may 2016. A total of 195 samples were screened for presence of Acinetobacter and Aeromonas by culturing on appropriate media. The isolates were identified via biochemical tests and confirmed by API 20E system which revealed Acinetobacter baumannii (15 isolates) and Aeromonas hydrophila (11 isolates). The sources of Acinetobacter were from urine (2 isolates), Wound (8 isolates) and burn (5 isolates). While, the sources of Aeromonas were from burn (1 isolate) and stool (10 isolates) .The results showed that ESβLs enzyme produced from(2) of Acinetobacter and (3) of Aeromonas .The results also showed high rates of resistance to amoxicillin, ticarcillin and carbencillin in both bacteria.*

KEYWORDS: ESβLs, *Acinetobacter baumannii, Aeromonas hydrophila*

INTRODUCTION

Extended spectrum β- lactamases (ESβLs), a type of β-lactamase enzymes (class A b-lactamases according to Ambler's classification), are have ability to hydrolyze penicillins, cephalosporins and monobactams, but not to cephamycins or carbapenems, and are inactivated by β-lactamase inhibitors. (Jacob and Munoz-Price,2005), and are encoded to it by mobile gene like plasmid…etc (Thompson,2010). These genes usually code resistance to several antibiotics including cephalosporins and other antibiotics like aminoglycosides, tetracyclines, fluoroquinolones and as well sulfamethoxazole-trimetroprim (Chatterjee *et al.*, 2012). ESβLs are capable of hydrolyzing extended spectrum cephalosporins with an oxyimino side chain. These cephalosporins like cefotaxime, ceftriaxone, ceftazidime and also cephpodoxime (Emery and Weymouth ,1997). Infections caused by ESβLs-producing bacteria like *Acinetobacter* , *Aeromonas* and of course other Gram negative are related with increased morbidity and mortality which is joined to inappropriate or delayed antimicrobial curing (Knudsen and Andersen,2014). *Acinetobacter baumannii* is involved in hospital outbreaks worldwide and is an opportunistic pathogen (Laurent *et al.*, 2003). In the last decades , *A. baumannii* has become more prevalent as opportunistic pathogen and an significant species has role in nosocomial infection, causing several infections including pneumonia, septicemia, urinary tract infections, and also wound infections(Perez *et al.*, 2007;Remy *et al.*, 2011). Lately, notes of strains of *Acinetobacter baumannii*, which

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are resistant to all known antibiotics, has increased, which suggest a boring and concern development that should be worked on quickly by the international health care community and local governments. While, another characteristic, alongside the increased resistance profile, which makes of *Acinetobacter baumannii* a oversetting pathogen, is its ability to survive for long periods of time, especially in hospital settings, thus increasing the ability for nosocomial spread (Peleg *et al*., 2008).

Reports of multidrug-resistant isolates has rising over the last years, which has in turn led to an increased use of broad-spectrum antibiotics (Laurent *et al.,* 2003) . Curing of infections due to this microbe poses a major clinical trouble (Peleg *et al* ., 2008). The most of the expanded-spectrum β-lactamases of *Acinetobacter* , *Aeromonas* and as well other Gram negative microbes are the clavulanic acid-inhibited extended-spectrum β-lactamases (ESβLs) of Ambler class A that has been notified extensively and are widespread (Nordmann,1998;Laurent *et al.,* 2003). As well as other important microbe is like *Aeromonas* species and too Gram-negative bacilli, is distributed globally and as well can grows ubiquitously in the natural environment. *Aeromonas* microbe as human pathogens has role of in natural disasters was supported by the observation that they ranked as the single most popular pathogen identified in people that tsunami survivors with skin or soft tissue infections in Thailand in 2004 (Hiransuthikul *et al.,* 2005).

Besides skin or soft tissue infections, *Aeromonas* can be cause a several of human diseases in the community or hospital settings, like gastroenteritis, abdominal/peritoneal sepsis, septicemia, hepatobiliary tract infections and as well catheter-related infections (Wu *et al* ., 2007; Janda and Abbott , 2010). Both immunocompromised and immunocompetent people would gain infections due to *Aeromonas* microbe, mostly from oral consumption of or direct mucocutaneous contact with contaminated water or foods by these microbe. (Janda and Abbott , 2010). *Aeromonas hydrophila*, *A. caviae*, and *A. veronii bv. sobria* are the three major *of Aeromonas* species appear to be relative with human diseases. (Janda and Abbott , 2010). *Aeromonas* can produce several of β-lactamases which give resistance to a broad spectrum of β-lactams (Tamar and Dennis , 2010). Three major classes of chromosomally mediated β-lactamasesd Ambler class B, C, and as well D β-lactamasesd has been noted in *Aeromonas* species. (Fosse *et al.,* 2003;Janda and Abbott , 2010). Metallo- β-lactamases (MBLs), AmpC β-lactamases, and penicillinases are the principal class B, C, and as well D β-lactamases found in *Aeromonas* microbe, respectively (Janda and Abbott , 2010) . Another related class of β-lactamases addressed is class A extended-spectrum β-lactamases (ESBLs), which has been increasingly notified in both clinical and environmental *Aeromonas* microbe (Girlich *et al.,* 2010;Wu *et al*., 2011).

The aim of the present study is to detect of Extended-Spectrum β-lactamases (ESβLs) enzyme and antibiotic profile including quinolones in *Aeromonas* and *Acinetobacter* isolated from clinical specimens from Hussein teaching hospitals and Public Health Laboratory in Thi Qar , Iraq.

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METHOD

In the present study samples collected from patients retened /or admitted to Hussein teaching hospitals and Public Health Laboratory in Thi-Qar province between November 2015 and may 2016. A total of 195 samples were collected including: wounds swabs (52) , burn swabs (56) ,urine samples (43) and stool samples (44).

Isolation and Identification of Bacterial Isolates

All specimens were cultured on blood agar and MacConkey agar and incubated overnight at 37°C under aerobic conditions. Depending on morphological of features of colonies and microscopical examination with Gram stain then biochemical tests were used to differentiate *Acintobacter* and *Aeromonas* from other gram negative bacteria. Diagnosis of species was confirmed by API 20E system.

ESBLs Detection

The presence of ESβLs was detected in all isolates using the double disc test (Briefly test). Organisms were emulsified in sterile water and the turbidity matched with 0.5 McFarland standards. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. A disc containing 30 µg amoxicillin–clavulanate was placed at the centre of the agar plate. A 30 µg ceftazidime disc was placed 25 mm from the amoxicillin–clavulanate disc and another disc containing 30 µg cefotaxime was placed on the opposite side of the amoxicillin–clavulanate disc (25 mm apart). The plates were incubated at 37 °C overnight and ESβLs production was inferred as positive if there was an expansion of the zone of inhibition clavulanate disc, cefotaxime and amoxicillin– clavulanate disc or both (Livermore and Brown,2001).

Antibiotic Testing

Susceptibility tests were performed on all bacterial isolates against 17 antimicrobial agent (Bioanalyse, Turkey) so from different classes have been determined depended on using kirby-Bauer disc diffusion method (Bauer *et al*.,1966). Inhibition zone around antibiotic discs was measured as found in CLSI guidelines (2014) . The agents tested included amikacin (AK: 30 µg), ceftriaxone (CRO: 30 µg), ciprofloxacin (CIP: 5 µg), gentamicin (CN: 10 µg), imipenem (IMP: 10 µg), meropenem (MEM: 10 µg), cefotaxime (CTX: 30 µg), ceftazidime (CAZ: 30 µg), amoxicillin (AX: 10 µg),amoxicillin - clavulanic acid(AUG:30 µg) norfloxacin (NOR:10 µg) ,naldixic acid (NA:30 µg) ,netilmicin (NET:30 µg) ,ticarcillin (TI:75 µg) , nitrofurantion (F:300 µg) , carbencillin (PY:100 µg) and aztreonam (AT:30 µg).

RESULTS

A total 195 samples of clinical samples were collected from Hussein Teaching Hospitals and Public Health Laboratory during the period November 2015 to may 2016 . *Acinetobacter baumannii* were isolated from (15) cases (2 urine, 8 wound, 5 burn) and *Aeromonas hydrophila* were isolated from (11) cases (1 burnand 10 stool) (Table 1). ESβLs producing were detected in 5 isolates, (2) as *Acinetobacter*

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baumannii one from urine and the other from wound and (3) as *Aeromonas hydrophila* all were from stool.

Table (1): Number and Percentage of clinical samples and also number of isolates that ESβLs production

Source of sample	No. of sample	No.(%) of *Acinetobacter spp*	No.(%) of *Aeromonas spp*	No. of positive *Acinetobacter* for ESβLs producing	No. of positive *Aeromonas* for ESβLs producing
Burn	**56**	**5 (8.92%)**	**1 (1.78%)**	**-**	**-**
Wound	**52**	**8 (15.38%)**	**-**	**1**	**-**
Urine	**43**	**2 (4.65%)**	**-**	**1**	**-**
Stool	**44**	**-**	**10(22.72%)**	**-**	**3**

This study showed high rates of resistance among *A. baumannii* isolates to cefotaxime, ceftriaxone, ticarcillin, amoxicillin and ceftazidim (Table 2). According to these results, most isolates were susceptible to netilmicin and meropenem with low resistance rates (Table 2).

Table 2. Antibiotics-susceptibility for *A. baumannii* isolates

Antibiotics	Susceptibility; no. (%) of isolates: Susceptible		Resistant	
Amikacin	7	(46.7)	8	(53.3)
Ciprofloxacin	5	(33.3)	10	(66.7)
Imipenem	6	(40)	9	(60)
Meropenem	8	(53.3)	7	(46.7)
Ceftriaxone	0		15	(100)
Gentamicin	2	(13.3)	13	(86.7)
Cefotaxime	0		15	(100)
Norfloxacin	4	(26.7)	11	(73.3)
Naldixic acid	3	(20)	12	(80)
Netilmicin	11	(73.3)	4	(26.7)
Ticarcillin	0		15	(100)
Carbencillin	1	(6.7)	14	(93.3)
Amoxicillin-clavulanic acid	2	(13.3)	13	(86.7)
Amoxicillin	0		15	(100)
Ceftazidim	0		15	(100)
Nitrofurantion	1	(6.7)	14	(93.3)

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Also this study showed that *Aeromonas hydrophila* have high rates of resistance to amoxicillin, amoxicillin- clavulanic acid, ticarcillin and carbencillin (Table 3). According to these results, most isolates were susceptible to netilmicin , ciprofloxacin and amikacin with low resistance rates of (9.1%), (9.1%) and (9.1%) respectively (Table 3).

Table 3. Antibiotics-susceptibility for *Aeromonas hydrophila* isolates

	Susceptibility; no. (%) of isolates:			
Antibiotics	**Susceptible**		**Resistant**	
Amikacin	10	(90.9)	1	(9.1)
Ciprofloxacin	10	(90.9)	1	(9.1)
Imipenem	5	(45.45)	6	(54.54)
Meropenem	6	(54.54)	5	(45.45)
Ceftriaxone	3	(27.27)	8	(72.73)
Gentamicin	9	(81.81)	2	(18.18)
Cefotaxime	3	(27.27)	8	(72.73)
Norfloxacin	9	(81.81)	2	(18.18)
Naldixic acid	8	(72.72)	3	(27.27)
Netilmicin	10	(90.9)	1	(9.1)
Ticarcillin	0		11	(100)
Carbencillin	0		11	(100)
Amoxicillin-clavulanic acid	0		11	(100)
Amoxicillin	0		11	(100)
ceftazidim	6	(54.54)	5	(45.45)

DISCUSSION

Treatment of infections caused by ESβLs enzyme producing *A. baumannii* and *Aeromonas hydrophila* has emerged as an important defiance. These organisms usually targets the immunocompromised and also most susceptible patients. Lately, infections by *Acinetobacter baumannii* which involve the central nervous system, soft tissue and skin (Peleg *et al*., 2008). Besides skin or soft tissue infections, *Aeromonas spp* can lead to cause a variety of human diseases in the community or hospital settings, such as gastroenteritis and septicemia. Both immunocompromised and immunocompetent individuals would acquire infections due to *Aeromonas*, *spp* usually from oral consumption of or direct mucocutaneous contact with polluted foods or water (Janda and Abbott, 2010). ESβLs enzyme producing strains have been widely reported all over the world, such as Palestine, Europe, North America, and China also reported of Iraq (Owlia *et al.,* 2012). The factors which increasing the number of isolates resistance were such that: Long-term hospitalization , use the last line drugs (including third-generation cephalosporins), transfer plasmids containing antibiotic resistance genes to susceptible isolates, stability of this resistant isolates by transmission of patient to patient. In our study, all isolates were *Acinetobacter baumannii* and *Aeromonas hydrophila* Meric et al. was similar with

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the study (Meric *et al.,* 2005). In our study showed that the highest number of *Acinetobacter baumannii* isolates from wound ,burn and urine respectively . In case *Aeromonas hydrophila* highest number from stool and burn respectively . Return to ESβLs enzyme producers two different studies in Korea and Turkey showed an incidence of 54.6% and 46% ESβLs producers, respectively (Yong *et al* ., 2003). Some studies showed that ESβLs enzyme producing strains could be carrying genes coding for resistance some antibiotics that using in Treatment of infections caused by ESβLs enzyme producing microbe (Bonnin *et al* ., 2011). therefore, genetic research will be needed for the detection of genes. This finding suggests that genes coding for ESβLs and genes coding for resistance to some antibiotics may reside within the same plasmids and therefore spread together.(Gouby *et al* ., 1992). Gram-negative bacteria, are adapted to exchanging genetic information and antibiotic resistance in these organisms is often due to the acquisition of genes from a shared pool (Iredell & Partridge, 2010). In our study showed that the highest resistance to quinolones antibiotic in *Acinetobacter baumannii* isolates from wound and burn. whilst *Aeromonas hydrophila* showed that the highest resistance to amoxicillin, ticarcillin and carbencillin and more susceptible to quinolones antibiotic.

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