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Short Communication

Study of ß-lactamases production from aerobic gram positive and negative bacteria causing bacteraemia in children in Baghdad

Raad Naji Hasan

Department of Biology, Faculty of Science, University of Al-mustansiriya, Baghdad, Iraq.

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A total of 200 blood samples were collected from children who had suffer from diarrhea, abdominal pain and fever above 38°C, which are of symptoms of bacteraemia, from Children hospital in Baghdad. The aims were isolation and identification of aerobic bacterial species causing bacteraemia in children and to determine antimicrobial susceptibility pattern of these isolates and their ability to produce extended spectrum ß-lactamases and their plasmid content. The results showed that 70 isolates (60.8%) belonged to *Staphylococcus* of which, 11 isolates (9%) *S. aureus* and 59 (51.3%) isolates were *S. epidermidis*; other bacteria were *P. aeruginosa* (7 isolates, 6%), *E. coli* (11 isolates, 9.5%), *Klebsiella* spp (14 isolates, 12.17%), *Acinetobacter baumani* (4 isolates, 3.4%), *Enterobacter* spp (5 isolates, 4.3%), *Streptococcus* spp (2 isolates, 1.7%), *Salmonella* spp (1 isolate, 0.8%) and *Brucella* spp (1 isolate, 0.8%). Sensitivity of the isolates was tested against 12 antibiotics. Results revealed variable in their sensitivity for antibiotics. Amikacin, imipenem and vancomycin were found to be the most effective agents against the isolates. On the other hand, the results showed that 30% of isolates were extended spectrum ß-lactamase producing and having plasmid with different sizes.

Key words: ß-Lactamase, aerobic bacteria, bacteraemia.

INTRODUCTION

Blood infection is a significant proportion of the total infection and total mortality in the world. It is among the most important cases related to health care (Charles et al., 2008). 250,000 cases of this kind are recorded in the United States annually (Ashare et al., 2006). These infections occur because of bacteria in the positive and negative Gram stain, but the positive bacteria of Gram stain is the most frequent cause of the blood infection (Abe et al., 2010). The skin and mucous membranes are of the most important sources of blood infection, so the infection comes from external or internal origin (Ashare et al., 2006). Surgery, diagnostic tools and therapeutic play an important role as factors causing blood infections (NNIS System, 1997). The production of ß-lactamases enzymes is one of the most important means of resistance of bacteria to antibiotics (Prescott et al., 2000). The seriousness of these enzymes have increased after the appearance of new varieties and more dangerous ones, such as extended-spectrum ß-lactamases enzymes (ESßLs) and metallo ß-lactamases (MßLs). The production of these enzymes consider as development in resistance to antibiotics in bacteria (Nordmann and Poirel, 2002). They give the bacteria, which produce them, the feature of antimicrobial resistance against wide spectrum ß-lactam which includes most of the new cephalosporins, arising out of these enzymes through mutations in the genes coded for ß-lactamases enzymes type TEM 1, TEM2 and SHV 1 (Paterson and Bonomo, 2005). CTX-M

E-mail: raadalhasani@yahoo.com. Tel: 00964 780 777 7728.

Table 1. Bacterial species that cause blood infection in children.

Isolated bacteria	Number of isolates	Isolating percent (%)
S. epidermidis	59	51.3
Klebsiellaspp	14	12.17
E. coli	11	9.5
S. aureus	11	9.5
P. aeruginosa	7	6
Enterobacterspp	5	4.3
Acinetobacter spp.	4	3.4
Streptococcus spp	2	1.7
Salmonella spp	1	0.8
Brucellaspp	1	0.8

enzymes are of the most common ESßLs enzymes in presently. They are extended-spectrum and highly effective against the anti-cefotaxime and cefotazdime encrypt by blaCTX-M gene which is on a conjugated large plasmid moving among different species of intestinal bacteria family. There are patterns of these enzymes most commonly; CTX-M15, CTX-M16, CTX-M8 and CTX-M14, according to some studies on the bacteria *E. coli*, *Enterobacter* and *Proteus mirabilis* (Naas et al., 2007).

The importance of this study is that the blood cultures samples are the most important samples up to the lab, as the samples of culture positive may indicate a direct threat to the lives of injured blood, and must be dealt with seriously and expeditiously in order to avoid the occurrence of death, so it is necessary and important to isolate the pathogenic bacteria, especially in children cases, and determine the types, and the types of antibiotics that affect them, and study the production of wide spectrum ß-lactamases enzymes.

MATERIALS AND METHODS

Sampling

200 blood samples have been collected from children who had suffer from diarrhea, abdominal pain and fever above 38°C less than 3 years in a hospital for the protection of children in Baghdad, for the period of 09/01/2010 until 01/02/2011, in order to determine aerobic bacterial species that cause infection to the blood. Blood samples between 5 to 10 ml were taken and put in a bottle of blood cultures of antenna implant. The bottle been kept in the incubator at a temperature of 37°C, and was monitored manually for a five days, we have identified whether it is positive or negative culture. Blood agar dishes planted and MacConkey and then incubated at a temperature of 37°C aerobically for 24 h in order to obtain pure bacterial colonies. Planted bacteria were subjected to bacterial microscopic and biochemical tests for diagnosis of bacterial isolates (Greenwood et al., 2002).

Test the sensitivity of isolates to antibiotics

Sensitivity of the isolates tested for antibiotic discs (Kirby Bauer method) using the center of AGAR Mueller Hinton note that the disks used were anti-life as follows: AX: Amoxycillin, KF: cephalothin, OX: oxacillin, FEP: cefepim, CRO: ceftriaxone, PRL: pepracillin, DO: doxycyclin, CTX: cefotaxime, GAT: gatifloxacin, VA: vancomycin, IMP: imipenem, AK: Amikacin.

A screening test for the production of extended-spectrum ß-lactamases enzymes

Extended spectrum ß-lactamase (ESBLs)

Disc approximation method was used to investigate the extended-spectrum ß-lactamases enzymes as used by Jarlier et al. (1988).

RESULTS

70 isolates (60.8%) belong to the *Staphylococcus* bacteria were obtained and distributed among 11 isolates (9.5%) was related to the type of isolation of *S. aureus*; 59 isolates (51.3%) belonged to type *S. epidermidis*, 7 isolates (6%) belonging to the bacteria *P. aeruginosa*, 11 isolates (9.5%) belonging to the bacteria *E. coli*, 14 isolates (12.17%) belonging to the bacteria *Klebsiella* spp, as well as the isolates (3.4%) for the bacteria *Acinetobacter baumanii*, 5 isolates (4.3%) of the bacteria *Enterobacter* spp, 2 isolates (1.7%) for bacteria *Streptococcus* spp, 1 isolate (0.8%) for the bacteria *Salmonella* spp and 1 isolate (0.8%) for the bacteria *Brucella* spp (Table 1). The other 85 samples were negative. Out of the 200 samples, there were 85 negative samples which were neglected.

DISCUSSION

The ratio of isolated *Staphylococcus* from positive blood cultures in our study was 60.8%. This high rate attracts the attention to the importance of these bacteria as a season of blood infection, whether it is real or not in children, in local hospitals, and it were higher than isolation ratio of bacteria-negative Gram stain. This result agree with the findings of researchers (Abe et al., 2010; Graninger and Ragette, 1992), who isolated positive-bacteria Gram stain in a higher ratio than bacteria-negative Gram stain (high ratio of *S. epidermidis* to the total number of bacteria *Staphylococcus*, may not be real ratio. This higher ratio reveals many non-real blood infections by these bacteria, as it is one of the most important pollutants from the skin flora. On the other hand, the highest percentage isolation of bacteria-negative Gram stain is a bacterium *Klebsiella* spp as isolated by 12.17%, followed by bacteria *E. coli* by 9.5%. This is consistent with the result of Thom et al. (2008) who were able to isolate these two types of blood infection in some hospitals in the United States of America, found (Watanakunakorn and Jura, 1991) the proportion of deaths bacteria *Klebsiella* spp causing blood infection of between 19 to 50%; our results do not agree with the result

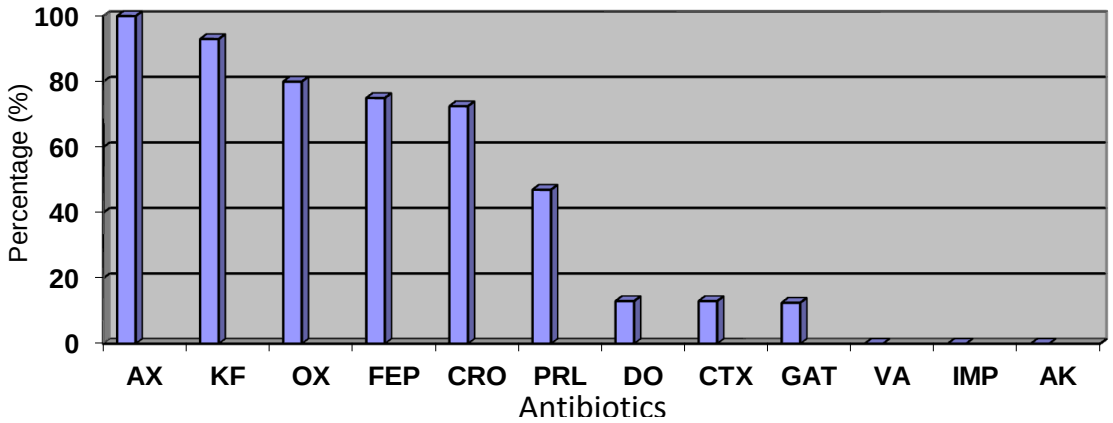

Figure 1. The percentage of resistant isolates, the study of antibiotic disk diffusion method.

of Wisplinghoff et al. (2004) who found that the percentage of deaths *E. coli* bacteria that cause blood infection is 20%, and were less ratio to isolate the bacteria, *Salmonella* spp and *Brucella* spp, and this is consistent with the result of Dhanoa and Fatt, (2009) who isolated a bacterium *Salmonella* spp by a few immunocompetent inhibited patients.

The results of examination of sensitivity to antibiotics show clear variance in resistance isolates to antibiotics used, and the anti-amikacin, Alombnim and vancomycin of more antibiotics effective against bacteria that cause infection vessels under study; in contrast, anti-amoxycillin and cephalothin less antibiotics effective against it (Figure 1).

Antibiotics

AX: Amoxycillin, KF: cephalothin, OX: oxacillin, FEP: cefepim, CRO: ceftriaxone, PRL: pepracillin, DO: doxycyclin, CTX: cefotaxime, GAT: gatifloxacin, VA: vancomycin, IMP: imipenem, AK: Amikacin.

The results of the current study, as in Figure 1 show that the best antibiotics in bacterial isolates their impact on the study is the anti-imipenem,anti-amikacin and anti-vancomycin, and this result is similar to the results of many researchers who believed that these antibiotics are the best treatments for blood infection (Guilfoile, 2007). In contrast, the anti-Amoxycillin and cephalothin Alawxaslin was less effective against isolates of the study, pointed out by Lauplend and Conly (2003) to increase the resistance of Staphylococcus bacteria to penicillins since the 1970 semi-manufactured private derivatives such as methicillin and oxacillin. A number of studies mentioned that these bacteria acquire an internal resistance to antibiotics cephalosporins, penicillins, the aztreonam, clindamycin and Co-Trimoxazoleas results of mutations at the site to effectively link counter, or mutations in proteins associated with penicillin PBPs most common in bacteria positive for Gram stain and ownership of genes jumping and production of ß-lactamases enzymes that may be chromosomal origin, plasmid or acquired resistance resulting from the movement of mobile genetic elements such as plasmids and other (Brooks et al., 2004; Lynett et al., 2004).

The results of the present study showed that 30% of the bacterial isolates have the ability to produce extended-spectrum ß-lactamases enzymes (ESβLs). The widespread use of cephalosporins antibiotics, such as Ceftriaxon and Cefotaxime led to the development of the production of CTX-M enzymes by pathogenic bacteria (Bonnet et al., 2004). Extended-spectrum ß-lactamases enzymes are important factors in the pathogenesis of bacteria that produce them, as it was found that patients infected with bacteria producing these enzymes prolonged period of stay in hospital, it was found (Anonymous, 2002) that the average stay of patients with bacteria *Klebsiella* spp producing these enzymes in the hospital after the injury for 29 days for 11 days for patients with the same bacteria is producing these enzymes. Many studies have pointed to the steady increase in the productivity of the bacteria to the intestinal enzymes family extended spectrum ß-lactamases in different geographical areas, and that the spread of antibiotic resistance increased in the intestinal bacteria producing these enzymes (Nijssen et al., 2004). The results showed possession of isolates producing ß-lactamases enzymes, a wide spectrum of different-sized plasmid packages after the removal of the resulting agarosegel concentration of 0.7% (Figure 2).

We recommend that it is necessary to do susceptibility testing of antibiotics per sample bacteria isolated from the blood before starting treatment which is of great importance in the selection of an antibiotic effective and appropriate, in order to avoid "treatment failure primarily that could kill the patient, and to avoid" the emergence of bacterial strains a new resistance to antibiotics when used indiscriminately and inappropriate, and add it to spare the

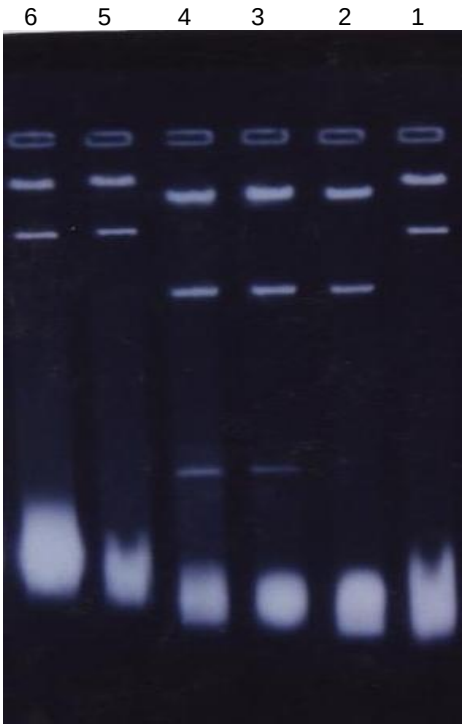

Figure 2. Plasmid content of the isolates which produce extended-spectrum ß-lactamases enzymes. (2 and 1), *E. coli* bacteria; (4, 5 and 3), bacteria Klebsiella spp.; (6), bacteria, Acinetobacter spp.

patient the cost of treatment of high-priced and a waste "material" to choose an antibiotic without the high cost and the expected effect.

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