The Study oF Resistance Pattern of Some Bacteria Isolated from Child Blood to Two Aminoglycoside Antibiotics

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Abstract

The present study was conducted to isolate pathogenic bacteria from the blood and to determine their resistance pattern with two aminoglycosides (gentamicin and amikacin). Two thousand four hundred and eleven blood samples were collected from children aged ≤ 15 years old who admitted to Kirkuk Pediatric General Hospital from Janurary2009 to August2010. The all rate of positive cases were 223 samples (9.2%) which included bacteremia in samples with the rate 83.4% for gram negative bacteria followed by 16.6% for gram positive bacteria. The following bacterial species were isolated: *E.coli* 91 (40.8 %), *Klebsiella spp*. 44 (19.7%) ,*Staphylococcus aureus* 34 (15.3%) , *Pseudomonas spp*. 20 (9%), *Proteus spp*. 20 (9%), *Salmonella spp*. 11 (4.9 %) , while *Enterococcus spp*. was found in only 3 culture (1.3%). The most common etiologic agents of pediatric bacteremia were *E.coli* and*Klebsiella spp*., together isolated from 60.5% of the blood samples studied. The rate of infection in males 58.8% was higher than in females 42.2% (P> 0.05).Considering the age groups the results showed that infants under one month of age were more susceptible to bacteremia than other age groups, in a rate of 114 (51.1%), but statistically the relationship between the infection and age was not significant(P> 0.05). The resistance rate of the isolated bacteria to gentamicin and amikacin was studied by disc diffusion method. The results showed the resistance rate of both gram-positive& gram –negative bacteria to amikacin were 8.1% &7.5%respectively lower than gentamicin 48.7%&25.3% respectively. Finally, amikacin was the most effective antibiotic than gentamicin among blood bacterial isolates.

Keywords: Bacteremia , Aminoglycosides, Bacterial resistance.

دراسة نمط المقاومة لبعض انواع البكتريا المعزولة من دم الاطفال لاثنين من المضادات الحيوية الامينوكلايكوسيدية

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الخلاصة

اجريت هذه الدراسة لعزل البكتريا الممرضة من دم الاطفال و تحديد نمط مقاومتها لاثنين من المضادات امينوكلايكوسيدية (Amikacin و Gentamicin). وقد تم جمع ٢٤١١ عينة دم من الاطفال ذو الاعمار ≤١٥ سنة والذين راجعوا مستشفى الاطفال كركوك العام خلال الفترة من كانون الثاني٢٠٠٩ الى اب٢٠١٠. وكانت نسبة الحالات الموجبة ٢٢٣ عينة (9.2%)وبنسبة ٨٣,٤ % لبكتريا السالبة الكرام و ١٦,٦ % لبكتريا الموجبة الكرام . و الانواع البكتيرية التي تم عزلها: بكتريا *E.coli* اعطت اعلى نسبة من بين مجموعة البكتريا المعزولة (٤٠,٨%) تلتها بكتريا*Klebsiellaspp* اعطت (١٩,٧%) ثم بكتريا *Staphylococcus aureus* (١٥,٣%) وبكتريا (*Pseudomonas spp* ٩%)وبكتريا *Proteus spp.* (٩%) و*Salmonella spp.* (٤,٩ %) واخيرا بكتريا *Enterococcus spp.* اعطت نسبة (١,٣ %). حيث شكلت *E.coli*و*Klebsiellaspp.*اعلى نسبة العزل(٦٠,٥%) من بين المجموعة البكتيرية المعزولة. معدل الاصابة في الذكور كانت ٥٨,٨% وهي اعلى مما في الاناث ٤٢,٢% و بدون وجود فروقات معنوية (p>0.05). مع الاخذ بنظر الاعتبار الفئة العمرية اظهرت النتائج ان الاطفال الرضع اقل من شهر هم الفئة الاكثر عرضة للاصابة بتجرثم الدم اذ بلغت نسبتهم 51.1% ولكن احصائيا العلاقة بين الاصابة والعمر لم تكن معنويا p< 0.05و عند دراسة معدل مقاومة العزلات البكتيرية لمضادين amikacin و gentamicin بواسطة طريقة نشر القرص.

اظهرت النتائج ان معدل المقاومة كل من الجراثيم الموجبة و السالبة الكرام للمضاد amikacinكانت (٨,١ %و ٧,٥% على التوالي) وهي اقل مقارنة مع مقاومة لمضاد gentamicin (٤٨,٧ % و ٢٥,٣ %بالتتابع). و اخيرا يعتبر المضاد amikacin اكثر فعالية ضد الجراثيم المعزولة من الدم مقارنة مع المضاد gentamicin.

كلمات الدالة: تجرثم الدم ، مقاومة البكتريا ،مضادات الامينوكلايكوسيدية

Introduction

Bacteremia is the presence of viable bacteria in the circulating blood and is a common cause of morbidity & mortality in children despite advance in antimicrobial therapy and supportive care[1], therefore, bacteremia continues to be a serious problem that needs immediate attention and treatment[2]. Illness is associated with bacteremia ranges from self –limiting infection to life threating sepsis that requires rapid and aggressive antimicrobial treatment, which is complicated by increasing worldwide antibiotics resistance[3]. Blood culture still remains the most practical and reliable method for detection of microorganisms in patient blood, although recently many advanced techniques such as nucleic acid probes and polymerase chain reaction (PCR) have been developed for diagnosis of blood stream infections[4].

The incidence of bacteremia in children varies widely. About 20-50% positivity has been reported by many workers][٥Also, the bacterial etiology of bacteremia varies widely from hospital to hospital and from one community to another; so also the antibiotic susceptibility pattern is influenced by location and time [6-8] .Bacteremia with gram- negative microorganisms is increasingly reported nowadays particularly in Asian countries[9].

The most frequent etiologic agents of bacteraemic cases include *E. coli*, *Klebsiellapneumoniae*,*Enterobacter spp.*, *Pseudomonas aeroginosa* and *Staphylococcus aureus*[10] . Children who younger than 36 months are at increased risk for bacteremia or sepsis secondary to the immaturity of their immune system[11].

Aminoglycosides are a class of antibiotics used extensively in clinical practice. However, their usefulness may be limited by the appearance of resistant strains. Various mechanisms are known to play a role in the development of aminoglycoside resistance but the presence of aminoglycoside modifying enzymes (AME) is the most clinical and epidemiological importance [12]. The genetic determinants of these enzymes are often located on Transposons facilitating the rapid dissemination of the genes in various bacterial populations.

The occurrence of these enzymes may show great geographic variability despite limited

geographic differences[13].Because bacteremia in children has different implications and different patterns than that in adult and the prevalence of aminoglycoside resistance among blood bacterial isolates has remained relatively low compared to other antimicrobial agents[14,15].Therefore, this study were aimed to isolate and identify the common pathogenicbacteria from blood culture of children in Kirkuk Pediatric General Hospital and their aminoglycoside (gentamicin &amikacin) resistance pattern with determination the relationship of the age &sex with incidence of bacteremia in children.

Materials &methods

Blood samples:

Two thousand four hundred and eleven blood samples were collected from infants &children clinically suspected bacteremia (aged ≤15years old) admitted to the Kirkuk Pediatric General Hospital as outpatients & inpatients for a period from January 2009 to August 2010.

Blood culture:

In all cases blood was obtained by peripheral venipuncture after clearing the skin by tincture of iodine then with 70% alcohol. Approximately 1 ml of blood was collected from neonates and 3 ml from children. These blood samples were introduced directly into Brain Heart Infusion (BHI) broth bottles, the ratio 1:10 of blood to medium wasused. Blood was mixed with 10 times of its volume with Brain-Heart Infusion (BHI) broth to dilute the effect of antibiotics (if taken) and to reduce the bactericidal effect of human sera. These cultures were incubated at 37˚C at least 7days [16].

Isolation & Identification:

All bottles were examined and positive blood culture broths identified by turbidity, hemolysis and pellicle formation. Three-subcultures were made at24, 72 hr. and on the 7th day at 37 ˚C on plates of MacConkey's agar, blood agar and Salmonella –Shigella agar .A negative result was followed up by examining the broth daily and doing a final subculture at the end of 7 days. The recovered bacterial isolates were identified by colonialcharacteristics, gram –staining and conventional biochemical tests [17, 18].

Aminoglycosides resistance pattern:

All isolates were tested for their resistance to Aminoglycoside antibiotics by using Kirby-Bauer disc diffusion method. Antibiotic discs used in this study were Amikacin

(AK)30µg & Gentamicin (G) 10µg. This test was performed on plates of Muller-Hinton agar. A bacterial suspension matching to 0.5 MacFarland suspensions was applied by sterile swab to the plates with leaved it 3-5 minutes at room temperature to dry. Antibiotic discs were placed on the agar with sterile forceps. The agar plates were incubated at 37°C for 18-24hr.Following incubation, inhibition zone sizes was measured with a ruler in millimeters[19,20].

Results & Discussion

Blood culture is the gold standard for the confirmation of bacteremia. The incidence of bacteremia in children varies widely[21].From 2411 blood culture samples of children ≤ 15 years of age were collected 223 (9.2 %) were culture positive (Table1).

Table (1): Number &Percentages of the positive and negative blood culture.

Blood culture	No.(%) of isolation
Positive	223(9.2%)
Negative	2188(90.8%)
Total	2411(100%)

In advance centers, the isolation rates vary from 6.7% to 55.4% [21],also another study reported that 16% of blood stream infections were occurred in children less than 15 years old [22].Our isolation rate 9.2% was agree with that recorded by Anbumani&Karki who stated that the rate of bacteremia among children were 7.9%, and4.2% respectively[23,24]. Comparatively, a high culture positivity rate has been reported by other investigators in other countries [25].Various factors affect the rate of isolation of organisms from the blood. These include the degree of bacteremia/prior antibiotic therapy, presence of fastidious organisms, the collection process, the ratio of the volumes of the amount of blood collected and liquid broth and finally the storage/delay prior to plating[21].

The rate of isolation was higher in males 129 (57.8%) as compared with females 94(42.2%) and the male to female isolate ratio (M/F) was 1.4:1, but there is no significant association between the sex & the incidence of the bacteremia $p>0.05$(Table 2).

Table (2): Frequency of occurrence of bacterial isolates (Based on sex).

Microorganisms	TotalIsolates	MaleNo. (%)	FemaleNo. (%)
Escherichia coli	91	53 (41.1)	38 (40.4)
Klebsiella spp.	44	28(21.7)	16 (17)
Staphylococcus aureus	34	20 (15.5)	14(14.9)
Pseudomonas spp.	20	12(9.3)	8(8.5)
Proteus spp.	20	8(6.2)	12(12.8)
Salmonella spp.	11	7(5.4)	4(4.3)
Enterococcus spp.	3	1(0.8)	2(2.1)
Total	223	129 (57.8)	94 (42.2)

P>0.05 non-significant

The predominance of bacteremia in male children was also observed with other studies [2, 25], but this predominance was not statistically significant; therefore this observation may be due to gender bias in presentation to hospital care.

Gram-negative bacteria constituted the major group of isolates 186/223 (83.4%) from bacteremic cases than gram positive were 37/223(16.6 %) as shown in table 3

Table (3): The percentages of microorganisms isolated from blood culture.

Microorganisms	No.(%)
E.coli	91(40.8)
Klebsiella spp.	44(19.7)
Staphylococcus aureus	34(15.3)
Pseudomonas spp.	20(9)
Proteus spp.	20(9)
Salmonella spp.	11(4.9)
Enterococcus spp	3(1.3)
Total	223(100)

This finding was in accordance with those found the predominance of gram negative bacilli which constituted 87.1% and 84.2% respectively of the total isolates[22,26].On the other

hand, these results were in contrast with that results being obtained by Berner and Colleagues who reported that the percentages of gram-positive and gram-negative bacteria isolated from

bacteremic children were 68% and 29% respectively[27]. Thus, predominance of either the gram-positive or gram-negative bacteria isolates is influenced by geographical location and changes in time.

E.coli was the predominant pathogen 91(40.8%) followed by *Klebsiella spp*. 44(19.7%), *Staphylococcus aureus*34 (15.3%), *Pseudomonas spp*. 20 (9%), *Proteus spp*. 20 (9%), *Salmonella spp*. 11(4.9%) and *Enterococcus spp*. 3(1.3%) as shown in Table3.Similar studies conducted by [10,28,29] which found that *E.coli* was isolated in 44.3%, *Klebsiella spp*. in 15% and *Staphylococcus aureus* 16.7% as the most pathogenic bacteria recovered from the blood samples. Also, the occurrence of *Pseudomonas spp.* , *Proteus spp., Salmonella spp. and Enterococcus spp.*were 9%, 9%, 4.9% and 1.3% respectively. Similar occurrence of these blood isolates was reported in different literatures[21,28,30].

The results showed the infants under one month of age (group A) was more susceptible to bacteremia with the rate of isolation 51.1% followed by children of age 1-12 month (group B) 28.7% and 20.2% from children age ≤ 15 years(group C)Table 4.

Table (4): Distribution the types of bacterial isolates in three age groups.

Bacterial isolates	Age groups No. (%)			
	Total isolates	Group A <1 mo.	group B 1 mo.-1 yr.	group C ≤15 yrs.
E. coli	91	48(52.7)	25(27.5)	18(19.8)
Klebsiella spp.	44	27(61.4)	13(29.5)	4(9.1)
Staphylococcus aureus	34	20(58.8)	10(29.4)	4(11.8)
Pseudomonas spp.	20	9(45)	9(45)	2(10)
Proteus spp.	20	9(45)	5(25)	6(30)
Salmonella spp	11	0	1(9.1)	10(90.9)
Enterococcus spp.	3	1(33.3)	1(33.3)	1(33.3)
Total	223	114(51.1)	64 (28.7)	45(20.2)

p>0.05 non-significant

The overall rate of isolation reduced with increasing the age. The data analysis showed no significant relation of bacteremia with age factor (p>0.05).The types of organisms cultured also

varied with age group. *E.coli,Klebsiella spp*. and *Staphylococcus aureus* were found highly prevalent among the age group belowone month (group A) by 52.7%, 61.4% & 58.8% respectively. In other hand, *Salmonella spp*. was isolated mostly in age group ≤15 year

(group C) by 90.9% (Table 4).This rate of isolation was higher than the other previous studies represented 33.9% and 25.7% [10,31].Prior investigators have reported high Susceptibility of infant to bacteremia than children may be due to immaturity in humoral , phagocytic and cellular immunity in neonates [32], beside that the early diagnosis of blood stream infection in infants is difficult because early symptoms of disease are non – specific [25].

E.coli ,Klebsiella spp. and *Staphylococcus aureus* found to be highly prevalent among newborns in our study, which was similar to the other study [25],but the higher isolation rate of *Salmonella spp*. was observed in children aged ≤15 (group C). This finding is in agreement with other study [٣٣].

Amikacin& gentamicin resistance of isolated organisms was also studied (Table 5).

Table (5): Resistance pattern of blood bacterial isolates to amikacin& gentamicin.

Microorganisms	Total isolates	Resistance to amikacin No. (%)	Resistance to gentamicin No. (%)
Gram-negative	186	14(7.5)	47(25.3)
E.coli	91	6(6.6)	٢١(٢٣,١)
Klebsiella spp.	44	4(9.1)	16(36.4)
Pseudomonas spp.	20	3(15)	6(30)
Proteus spp.	20	0	3(15)
Salmonella spp.	11	1(9.1)	1(9.1)
Gram-positive	37	3(8.1)	18(48.7)
Staphylococcus aureus	34	2(5.9)	18(52.9)
Enterococcus spp.	3	1(33.3)	0

Despite widespread use of aminoglycosides, development of resistance to this class of antibiotics remained low compared to other antimicrobial agents[14]. In our study,Amikacin resistance rate among gram-negative and gram positive blood stream isolates were 7.5% and 8.1 % respectively, while the gentamicin resistance rate were 25.3% & 48.7% alternatively.This

observation was confirmed with the results reported that the majority of gram negative blood stream isolates were susceptible to amikacin 90%[34].Accordingly, in a study from USA, amikacin resistance rate 11.3%-15.7% was significantly lower than

gentamicin 44.4%-51.9% [35].Also, in the another study in Turkey found higher resistant rate 54% of gram negative bacilli to gentamicin, and only 0.9% to amikacin[6].

Lower resistance rate to amikacin was observed among *Proteus spp.* (0%),followed by *E.coli* (6.6%) , *Staphylococcus aureus* (5.9%) and *Klebsiella spp*.(9.1%).While, these microorganisms showed to be high resistance to gentamicin and highly resistant rate was observed in *Staphylococcus aureus* isolates (52.9%) and *Klebsiella spp*.(36.4%). Among the *pseudomonas* isolates 15 % were resistant to amikacin and 30% resistant to gentamicin. 9.1% resistance rate was seen among *Salmonella* isolates towards both the amikacin& gentamicin. Additionally, One strain of *Enterococcus* (33.3%) showed resistant to amikacin and none of them resistance to gentamicin(Table5).This is in agreement with other studies which revealed more or less the same resistance rate[21,23]and highly resistance rate to gentamicin was observed by *Staphylococcus aureus* (52.9%)and *Klebsiella spp*.(36.4%). Similar findings have been reported elsewhere [7,8], amikacin& gentamicin resistance rates of *Pseudomonas spp.* were 15% &30% respectively. Monir*iet al.*reported similar findings, in which *Pseudomonas spp.* resistance to amikacin 17% was lower than gentamicin 31%[36]. Finally, 33% of *Enterococcus spp.* isolates showed resistant to amikacin, this result was higher than that reported by Roy*et al.*[37]. Inpatients blood stream isolates showed a higher degree of resistance towards both amikacin and gentamicin were accounted 9.02% &30.1% respectively than outpatient's bacteremia 5.6%&27.8% (Fig.1).Resistance against gentamicin has recently increased may be due to over-use or miss use of this antibiotic in both inpatients and outpatients acquired infection [2,14].

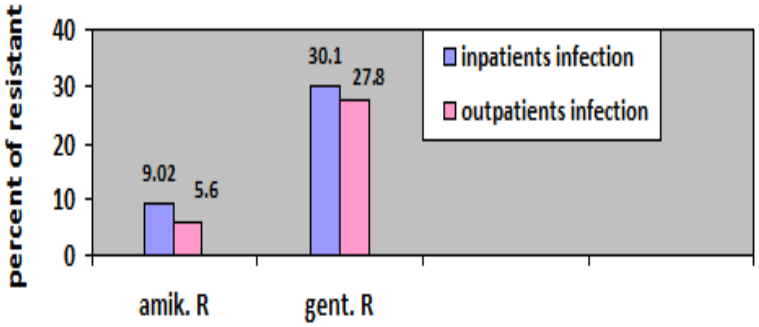

Fig.(1): Resistance To Aminoglycosidies Of Inpatients And Outpatients Bactetremia.

Conclusion

E.coli was found it's the predominate causative agent of bacteremia in children andanalysis of correlationbetween the age groups &gender with the rate of bacteremia showed no significant association (P>0.05). The resistance rate of gram-negative &gram-positive bacteria isolated from blood to amikacin was lower than gentamicin.

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