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In-Vitro Activity of Amoxycillin / Clavulanic Acid against Clinical Isolates from Jordanian Children.

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ABSTRACT

The extensive use of the β-lactam antibiotics in combination with β-lactamase inhibitors in hospitals and in the community has created major resistance problems which have led to increased morbidity, mortality and healthcare costs. To evaluate the in-vitro activity of Amoxycillin/Clavulanic Acid against clinical isolates from children. A total of 3315 bacterial pathogens were isolated and identified from various clinical specimens. These included 1590(47.9%) *Escherichia coli,* 599 (18.2%) *Klebsiella spp*, 77 (2.3%) *Proteus spp*, 97 (2.9%) *Pseudomonas aeruginosa*, 714 (21.5%) *Staph. Aurous*, 154 (4.7%) *Strepto coccus* and 84 (2.5%) *Enterobacter***.** The highest susceptibility rate of Amoxycillin/Clavulanic Acid was 83.7% for *Streptococcus* isolates, whereas the lowest susceptibility rate (11.3%) was recorded for *Pseudomonas aeruginosa*. The entire organisms showed low susceptibility to Amoxicillin/clavulanate except *Streptococcus spp* (83.7%). The activity of AMC against all the isolates of organisms fell far above the acceptable levels indicating wide spread and inappropriate use of AMC in pediatric infections. So it is the time to think, plan and formulate a strong antibiotic policy to address this present scenario

Keywords: Amoxycillin/Clavulanic Acid, *Escherichia coli*, *Klebsiella pneumoniae*, antimicrobial susceptibility, empiric therapy.

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INTRODUCTION

Antimicrobial resistance is a major public health problem worldwide, and several reports suggest that it is an increasing problem of phenomenal proportions, affecting both developed and developing countries. [1] Infections caused by resistant bacteria have been shown to be more frequently associated with increased morbidity and mortality than those caused by susceptible pathogens. [2,3] In areas of concentrated use, such as hospitals, this had led to lengthened hospital stays, increased health care costs and in extreme cases, to untreatable infections.

Among the large number of antibiotics being used, β-lactams agents are accounting for over 50% of all systemic antibiotics in use. [4]

Amoxicillin/clavulanate (AMC) was originally developed to extend the antibacterial spectrum of amoxicillin to include β-lactamase-producing species. [5]

Amoxicillin/clavulanic acid is a broad spectrum antibiotic consisting of amoxicillin trihydrate, a ß-lactam antibiotic, and potassium clavulanate, a ß-lactamase inhibitor. This combination results in increasing an antibiotic activity and restored efficacy against amoxicillin-resistant bacteria that produce ß-lactamase.

The most common cause of bacterial resistance to β-lactam antibiotics is the production of both β-lactamases and Extended Spectrum β-Lactamases (ESBLs). These enzymes are commonly produced by different bacteria especially *Escherichia coli* and *Klebsiella pneumoniae* and efficiently hydrolyze oxyimino-cephalosporins conferring resistance to third-generation cephalosporins such as cefotaxime, ceftazidime and ceftriaxone and to monobactams such as aztreonam. [6] Organisms producing these beta-lactamases may also be resistant to quinolones and aminoglycosides by different mechanisms. [7]

Despite world-wide use of β-lactam antibiotics, the distribution of the enzymes responsible for resistance to oxyimino-cephalosporins and cerbapenems is far from being uniform. Some hospitals in the United States seem to have no or low ESBLs, whereas in other hospitals, as many as 40% of *K. pneumoniae* isolates have been reported to be ceftazidime resistant as a result of ESBLs production. [8]

However, there is little information on Amoxicillin/clavulanic acid resistance pattern in Jordan. Therefore, this retrospective study was conducted to determine the rate of resistance to Amoxicillin/clavulanic acid by pathogens isolated from cultures of clinical specimens received from children inpatient and outpatients at Princess Rahmah Hospital during a period of 5 years (2005-2009).

MATERIALS AND METHODS

This study was carried out in the diagnostic Medical Microbiology Laboratory of Princess Rahmah Hospital located in Irbid, Jordan, between 2005-2009. A total of 3315 bacteria isolates were identified from different clinical specimens using standard bacteriological methods. These clinical specimens included blood, urine, ear swabs and conjunctival swabs. Microbiological and antibacterial susceptibility data of this study obtained from records of diagnostic Medical Microbiology Laboratory of Princess Rahmah Hospital. These data were filled in a prepared data sheet.

The antimicrobial susceptibility patterns of these isolates to antibiotics were determined using the Kirby-Bauer method of disc diffusion test. [9] Study protocol was approved by the Ethics Committee of the ministry of health in Jordan (MOH, REC, 08, 0057).

Statistical Analysis

Data were analyzed using SPSS (version15 for Windows) to calculate the frequencies and cross tabulation.

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RESULTS

A total of 3315 bacterial pathogens were isolated and identified from various clinical specimens. These included urine 2250 (67.8%), blood 510 (15.4%), ear swabs 463 (13.9%), conjunctival swabs 92 (2.9%) (Table1).

Types of isolates were 1590(47.9%) *Escherichia coli,* 599 (18.2%) *Klebsiella* species, 77 (2.3%) *Proteus* species, 97 (2.9%) *Pseudomonas aeruginosa*, 714 (21.5%) *S. Aurous*, 154 (4.7%) *Strepto coccus* and 84 (2.5%) *Enterobacter* (Table 2).

In vitro activity of Amoxycillin/Clavulanic Acid antibiotic against different bacterial isolates is illustrated in Table 2 and figure 1.

The highest susceptibility rate was 83.7% for *Streptococcus* isolates, whereas the lowest susceptibility rate (11.3%) was recorded for *Pseudomonas aeruginosa*.

In comparison between the year of 2005 and 2009, activity of Amoxycillin/Clavulanic antibiotic for all pathogenic isolates was significantly (P<0.05) decreased, It's also high significantly (P<0.001) decreased against *Klebsiella* and *Enterobacter*, where as the changing in the activity for other pathogenic isolates was not significant (Table 2).

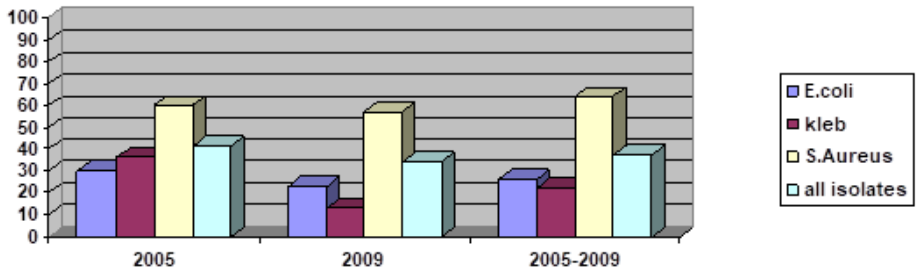
Table 1. Distribution of pathogens isolated from clinical specimens (2005-2009)

Sample	Year					Total
	2005	2006	2007	2008	2009	
Urine	192	449	546	470	593	2250
Blood	24	50	24	40	372	510
Ear	39	80	110	133	101	463
Eye	8	17	29	29	9	92
Total	263	596	709	672	1075	3315

Table 2. Amoxycillin/Clavulanic Acid activity to different bacterial pathogen isolates

Pathogen	2005	2006	2007	2008	2009	Total	Significance 2005 vs. 2009
	N (S %)	N (S %)	N (S %)	N (S %)	N (S %)	N (S %)	P-value
E-coli	132 (30.3)	305 (26.8)	383 (28.7)	361 (27.9)	409 (22.9)	1590 (26.8)	0.091
Klebsiella	41 (36.5)	108 (28.7)	132 (27.2)	111 (22.5)	207 (13.5)	599 (22.5)	<0.001
Proteus	9 (66.6)	24 (58.3)	19 (52.6)	5 (80.0)	20 (50.0)	77 (57.1)	0.422
Pseudo	15 (20.0)	14 (0)	25 (8)	26 (19.2)	17 (5.8)	97 (11.3)	0.242
S. Aureus	43 (60.4)	89 (67.4)	99 (69.6)	142 (75.3)	341 (57.4)	714 (64.1)	0.709
Strep. spp	12 (91.6)	39 (76.9)	36 (88.8)	24 (91.6)	43 (79.0)	154 (83.7)	0.326
Enterobacter	11 (72.7)	17 (47.0)	15 (40.0)	3 (100.0)	38 (13.1)	84 (35.7)	<0.001
All pathogens	263 (41.4)	596 (37.7)	709 (37.3)	672 (39.7)	1075 (34.2)	3315 (37.2)	<0.05

Figure 1. Susceptibility of clinical isolates to Amoxycillin/Clavulanic Acid (2005-2009).



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DISCUSSION

Results of this study showed that a total of 3315 pathogenic bacteria were isolated during the study period. The isolates were 1590(47.9%) *Escherichia coli,* 599 (18.2%) *Klebsiella* species, 77 (2.3%) *Proteus* species, 97 (2.9%) *Pseudomonas aeruginosa*, 714 (21.5%) *Staph. Aurous*, 154 (4.7%) *Strepto coccus* and 84 (2.5%) *Enterobacter*.

Amoxicillin/clavulanic acid (AMC) is widely used to treat many infections caused by susceptible bacteria, such as urinary tract infections, respiratory tract infections.

Results of this study demonstrate that AMC had poor activity against *E .coli* isolates through out the study period which was 26.8%. This results is consistence with results reported in India [10,11], whereas the AMC activity was lower than that reported in Jordan [12], Saudi Arabia [13], Spain [14], and Kenya [15]. However, in comparison of the activity of AMC against *E. coli* between the year 2005 and 2009 data showed decreased in the activity from 30.3% to22.9%, but this decrement was not significant.

Amoxycillin/clavulanic acid had also showed significant gradual decrement ($P<0.001$) in its activity against *Klebseilla pneumoniae* with activity rate of 36.5% to 13.5% in the years of 2005 and 2009 respectively. However, the main rate of activity for the five years of study period was 22.5 % which was lower than that reported in India. [10]

In this study, although moderate susceptibility rate (64%) of *S. Aureus* to AMC, it has remained almost unchanged over the period of study. Anyway, susceptibility rate was lower than that reported in Jordan [12] and higher than that reported in India. [16]

The entire organisms however, showed low susceptibility to Amoxicillin/clavulanate except *Streptococcus spp* (83.7%), and the susceptibility decrements was significant ($P<0.05$) in comparison between the year of 2005 and 2009. This finding is concordance with other findings reported higher resistance of Amoxicillin/clavulanate among both gram negative and gram positive organisms.[17]

The reason for the low activity of Amoxycillin/clavulanic acid against the tested isolates in this study could be attributed to the fact that Amoxycillin/clavulanic acid was a more commonly utilized empirical agent in Jordan. However, after the passage of time, different factors are attributable for emergence of resistance. These mainly include; high consumption of antibiotics, irrational use, incomplete course of therapy, and self-medication by patients, leading to the emergence of resistance and even treatment failures.

CONCLUSION

The present situation is alarming, because it is not long before Amoxycillin/clavulanic acid, an effective antibiotic would be failed to treat even simple or minor infections. In addition to this, routine antimicrobial susceptibility testing must be timely performed to determine the current status of resistance against antimicrobial agents. Otherwise therapy failures may occur which increase the cost of the therapy as well as recovery time from the underlying disease.

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