Antibiotic Susceptibility of Enterobacteria Isolated from One Hospital in Hilla, Iraq

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Abstract

A total of 153 isolates of enterobacteria were recovered from 187 clinical samples in one hospital In Hilla, Iraq during a period of three months. Enterobacteria isolates were mainly recovered from urinary tract (26.7%) and wounds (17.6%) samples.

The following bacterial species were recovered: *Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Klebsiella spp. Proteus mirabilis, P. vulgaris, Proteus spp., Enterobacter cloacae, E. aerogens, Enterobacter spp.,* and *Salmonella spp., and Citrobacter frundii. E. coli* was prominent in urine and stool, *Klebsiella* in wounds, and *Proteus* in urine and ear samples.

A standardized method was used to determine the susceptibility of enterobacteria isolates against 14 antibiotics. More than half of isolates were susceptible to penicillins (Amk, Amx), third generation cephalosporin (Ctx), and Rifadin (RD), but they were less susceptible to other penicillins (Pen, Amp), Gentamycin, and tetracycline.

Klebsiella pneumoniae isolates showed multiresistance to penicillin (100%), cephalexin, Gm, Te, RD, My, and NA. In addition to these antibiotics, half of the isolates showed resistance to extended spectrum β-lactam antibiotic cefotaxime.

Proteus mirabilis has the highest number of multi-resistant isolates. They were highly resistant to Pen, Gm, and showed moderate resistance to Amp, cephalexin, St, Te, and My antibiotics. High percentage of *Enterobacter* isolates were susceptible to all antibiotics used in this study except for P, Amp, and St.

Key words: Antibiotic susceptibility, Enterobacteria, hospital, bacterial infections.

الخلاصة

تم في هذه الدراسة عزل 153 عزلة من البكتريا المعوية من 187 عينة سريرية من مستشفى واحدة في مدينة الحلة/ العراق خلال فترة ثلاث اشهر. عزلت البكتريا بصورة رئيسية من عينات المجاري البولية والجروح. تم عزل الانواع البكتيرية التالية:-

Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Klebsiella spp. Proteus mirabilis, P. vulgaris, Proteus spp., Enterobacter cloacae, E. aerogens, Enterobacter spp., and *Salmonella spp., and Citrobacter frundii*

كانت عزلات *E. coli* هي السائدة في عينات الادرار والخروج وعزلات *Klebsiella* سائدة في عينات الجروح اما عزلات *Proteus* فكانت سائدة في عينات الادرار ومسحات الاذن.

استخدمت طريقة قياسية لتحديد حساسية العزلات ضد 14 مضاداً حيوياً. كانت اكثر من نصف العزلات حساسة للـ (Amk, Amx) السيفوتاكسيم والريفانين، غير انها كانت اقل حساسية تجاه البنسلينات الاخرى (البنسلين والامبسلين) والجنتاميسين والتتراسايكلين.

اظهرت عزلات *Klebsiella pneumoniae* مقاومة متعددة ضد البنسلينات cephalexin, Gm, Te, والريفانين.NA فضلاً عن ذلك كانت هذه العزلات مقاومة لمضادات البيتالاكتام واسعة الطيف (السيفوتاكسيم).

كانت عزلات *Proteus mirabilis* هي الاكثر عدداً بين العزلات التي اظهرت مقاومة متعددة. كانت نسبة عالية من عزلات *Enterobacter* حساسة لجميع المضادات المستخدمة في هذه الدراسة ماعدا مضادات P, Amp, and St

Introduction

Gram-negative rods belonging to the family Enterobacteriaceae are common agents of infections in hospitalized patients. They cause a large proportion of the cases of community and hospital-acquired bacteremia (Geerdes, *et al.*, 1992; Al-Charrakh, 2000; Al-Charrakh, *et al.*, 2005) and majority of cases of hospital-acquired pneumonia (Craven, *et al.*, 1991; Schaberg, *et al.*, 1991; Javris and Martone, 1992),

both being severe infections associated with a high mortality (Davies, 1996; MacFaddin, 2000).

The of bacterial strains capable of causing infections is increasing, and many of the them are resistant to one or more of the antibiotics used in therapy and this resistance constitute an increasingly serious threat to the current antimicrobial agents therapy (Jones, 2001).

The high level of resistance to antimicrobial agents shown by gram-negative rods is well-known and has been reported with increasing frequency (Jacoby, 1996; Jarlier, *et al.,* 1996; Al-Charrakh, 2000). The most important gram-negative resistance problems that impact on nosocomial infections are extended spectrum β-lactamases (ESBLs) in *Klebsiella pneumoniae, E. coli,* and *Proteus mirabilis* in addition to high level third generation cephalosporin (Amp C) β-lactamase resistance among *Enterobacter* and *Citrobacter frundii* (Jones and Pfaller , 1996).

The acquisition of resistance to antimicrobial agents may be due to chromosomial mutation or plasmids that are capable of transfer from one strain of bacteria to another, even across the species barrier (O'Brien and Acar, 1987; Bryan, 1989; Gold, 1996). Intergeneric spread of plasmids origination from multiresistant K. pneumonia or Serratia marcescens has frequently occurred among the enteric bacteria in hospitals (John and Twitty , 1986, Sirot, *et al.,* 1988).

Also the resistance genes are found on mobilized genetic elements called transposons (de la Cruz and Grinsted, 1982; Davies, 1996). Some transposons or plasmids have genetic elements termed integrons that enable them to capture exogenous genes. A number of genes may therefore be inserted into a given integron , resulting in resistance to multiple antimicrobial drugs (Levesque, *et al.,* 1995).

Since the outcome of severe infections caused by Enterobacteria may depend on rapid and appropriate therapy, and the choice of antibiotic is often based on the knowledge of the susceptibility profiles of the bacteria most commonly encountered. This study was conducted to evaluate the susceptibility and resistance of the enterobacteria isolated from clinical samples to a selected number of antibiotics.

Materials and Methods

Clinical samples:

Clinical samples were routinely obtained from inpatients (aged from 12 to 62 years) admitted to Hilla teaching hospital over a period of three months from September to November 2004.

A total of 187 clinical samples were examined for Enterobacteriaceae isolates. Samples included: 49 (26.2%) urine, 31 (16.5%) stool, 17 (9%) blood, 3 (1.6%) sputum, 10 (5.3%) pus, 9 (4.8%) aspiration, 24 (12.8%) swabs from upper respiratory tract (ear and throat), 29 (15.5%) wound swabs, and 13 (6.7%) skin swabs. Only bacterial isolates grown on MacConkey agar were considered in this study. Clinical samples that revealed no growth on MacConkey agar were neglected. For the sake of simplicity, which has separate isolates, was considered as two samples.

Bacterial isolates and Susceptibility tests:

Enterobacteriaceae isolates were identified to the level of species by using conventional biochemical tests (MacFaddin, 2000; Barrow, and Feltham; 2003) then the identification was confirmed using API systems strips as recommended by Biomérieux (France).

The susceptibility of the bacterial isolates to antimicrobial agents was determined by using disk diffusion method (NCCLS, 2003) and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) documents

(NCCLS, 2002). The following antimicrobial agents were obtained (from Oxoid, U.K.) as standard reference disks as known potency for laboratory use: Penicillin (P), Ampicillin (Amp), Ampiklox (Amk), Amoxicillin (Amx), Cephalexin (Cf), Cefotaxime (Ctx), Gentamycin (Gm), Streptomycin (St), Erythromycin (Er), Chloramphenicol (C), Tetracycline (Te), Lincomycin (My), Rifadin (RD), and Nalidixic acid (NA).

All these tests were performed on plates of Muller- Hinton agar (Oxoid, U.K.).

A 0.5 MacFarland suspension (provided by Biomérieux/ France) of tested bacterial isolates was applied to the plates , which were dried in an incubator at 35 ºC for 15 minutes . Antimicrobial disks were placed on the agar with sterile forceps .The agar plates were incubated inverted at 35 ºC for 18 hours. Results were recorded by measuring the inhibition zone (in millimeters) and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) documents (NCCLS, 2002).

Results

Clinical samples and Enterobacteria isolates:

A total of 146 clinical samples were positive for Enterobacteria growth giving a proportion of 78% of all 187 clinical samples studied. Of these, 19 samples were found to have mixed growth of two bacterial species, 7 of them were found mixed of two Enterobacteria species, and 12 of them were mixed of one Enterobacteria species and other of non-enterobacteria.

A total of 153 isolates of Enterobacteria were recovered from the clinical samples. The following bacterial genera were isolated: *Escherichia* (39.8%), *Klebsiella* (24%), *Proteus* (26.7%), *Enterobacter* (7.1%), *Salmonella* (1.3%), and *Citrobacter* (0.65%).

It must be pointed out that the frequency of the most common species found differed according to the type of the sample. *E. coli* was more frequent in urine and stool. *Klebsiella* was more frequent in wounds than in urine and stool samples. Proteus was prominent in urine and ear samples (Table-1). Differences in distribution according to the type of samples were much less marked for *Enterobacter* isolates.

In general, Enterobacteria were isolated much frequently from urine (26.7%) and wounds (17.6%) but they were less frequently isolated from sputum(0.6%) and throat (1.3%) samples (Figure-1).

Antibiotic susceptibility of Enterobacteria isolates:

More than half of *E. coli* isolates were susceptible to penicillins (Amk, Amx), third generation cephalosporin (Ctx), and Rifadin (RD), but they were less susceptible to other penicillins (P, Amp), Gentamycin, and tetracycline (Table-2). 57% of *Klebsiella* isolates were susceptible to Amk and Rifadin, but more than half of them were resistant to the rest of the antibiotics tested.

Klebsiella pneumoniae isolates showed multiresistance to penicillin (100%), cephalexin, Gm, Te, RD, My, and NA. In addition to these antibiotics, half of the isolates showed resistance to extended spectrum β-lactam antibiotic cefotaxime.

K. oxytoca isolates showed high resistance to P, Gm, Te, and My antibiotics. Half of them were less susceptible to the rest of the antibiotics used including cephalosporin

More than 50% of *Proteus* were susceptible to RD, Amk, Amx, and Ctx. They also showed high resistance to the other penicillins, cephalexin, Gm, St, Te, and My. *Proteus mirabilis* has the highest number of multi-resistant isolates. They were highly resistant to Pen, Gm, and showed moderate resistance to Amp, cephalexin, St, Te, and My antibiotics (Table-2).

High percentage of *Enterobacter* isolates were susceptible to all antibiotics used in this study except for P, Amp, and St. E. cloacae showed resistance to (P, Amp), but they were susceptible to the rest of the antibiotics tested.
Two isolates of *Salmonella* spp. were resistant to penicillin, and one of them showed multiresistance to most of the antibiotics used including chloramphenicol. *Citobacter frundii* isolate was susceptible to most of the antibiotics tested except P, Amk, Te, and NA.

Discussion

The result of the present study showed that *E. coli* isolates were the most frequent bacterial isolated from clinical samples and this result in agreement with (Jarlier, and Philipon, 1996). Differences in distribution of Enterobacteria according to the type of samples were detected in species of *E. coli, Klebsiella, Proteus* and this finding is similar to that obtained by several authors (Shah, *et al.,* 1991; Verbist, 1991; Jarlier, *et al.,* 1996).

E. coli and *Proteus* were most frequently isolated from urine samples (Table-1). This result was expected because these organisms are considered as the most etiologic agents of urinary tract infection in human.

The results also showed that Enterobacteria were resistant to most of penicillins studied and this attributed to their ability to produce β-lactamases enzymes. Resistance to beta-lactam antibiotics in Gram-negative bacteria can be due to three mechanisms: decreased permeability of the drug into the cell, hydrolysis of the drug by β-lactamase, or decreased affinity of the target penicillin-binding proteins-PBPs (Piddock, *et al.,* 1997).

The major mechanism of resistance in bacteria causing clinically significant infections remains the expression of β-lactamases, of which there are several classes including plasmid-encoded and chromosomally-encoded enzymes (Bush, *et al.,* 1995).

Klebsiella, E. coli, and *Enterobacter* are of increasing importance, as resistance to newer beta-lactams particularly extended-spectrum beta-lactams may be acquired by mutation in addition to plasmids (O'Brien and Acar, 1987; Moland and Thomson, 1994; Chanal, *et al.,* 1996, **Sirot**, 1995; Piddock, *et al.,* 1997). Our results showed, in addition to penicillins, multi-resistance among enterobacteria species to most of the antibiotics used in the present study. Multi-resistance patterns among Gram-negative rods particularly Enterobacteria has been reported frequently by many authors (Shah *et al.,*1991; Verbist, 1991; Jarlier, *et al.,* 1996).

Result of the study about susceptibility of *E. coli* isolates showed that these isolates were multiresistant to P, Amp, Gm, and Te (Table-1). The multi-resistance of *E. coli* isolates to most of the antibiotics used in the present study has been reported by several investigators (Al-Muhana, and Al- Charrakh, 1997; Al-Charrakh, 2000). *Klebsiella pneumoniae* isolates showed multi-resistance to P, Cf, Gm, Te, My, and NA. In addition to these antibiotics, resistance to extended-spectrum beta-lactams (Ctx). These results are in agreement with those isolates reported by several authors (Sirot, *et al.,* 1988; Tenover, 1991; Thomson, *et al.,* 1991; Al-Charrakh, 2000; Al-Charrakh, *et al.,* 2005).

Reported outbreaks of infection (Rennie, and Duncan, 1977; Courtney, *et al.,* 1980; Casewell, *et al.,* 1981; Facinelli, and Calegari 1984) caused by multiresistant strains of *K. pneumoniae* were associated with transferable resistance to gentamycin and cephalothin. The transfer of resistance among different genera of gram-negative

and between species enterobacteria has been reported by several authors (John and Twitty, 1986; Sirot, *et al.,* 1988.; Mazodier, and Davies, 1991 ;Courvalin., 1994).

Our results also revealed that half of *K. oxytoca* isolates showed resistance to most of the antibiotics used in the present study including cephalosporins and more than half of them showed resistance to P, Gm, Te, and My antibiotics (Table-2). The emergence of chromosomally encoded resistance to cephalosporins has been reported by strains of *K. oxytoca* (Sirot, *et al.*, 1988; Then, *et al.*, 1983).

Proteus mirabilis has the highest number of multi-resistant isolates. This findings are similar to those obtained by Jarlier et al (1996). High percentage of *Enterobacter* isolates was susceptible to all antibiotics used in this study except for P, Amp, and St. This result in unlike to several clinical strains reported by other investigators (Shah, *et al.*, 1991; Verbist, 1991; Jarlier, *et al.*, 1996; Al-Charrakh, 2000) that showed multiresistance to several antibiotics.

The low number of *Salmonella* and *Citrobacter* isolates in this study is attributed to that these organisms are less encountered in clinical samples if compared to other members of Enterobacteriaceae. The multiresistance of *Citrobacter frundii* isolates to several antibiotics was reported in other study (Jarlier, *et al.*, 1996), although our isolate showed susceptibility to most of the antibiotics used in this study (Table-2).

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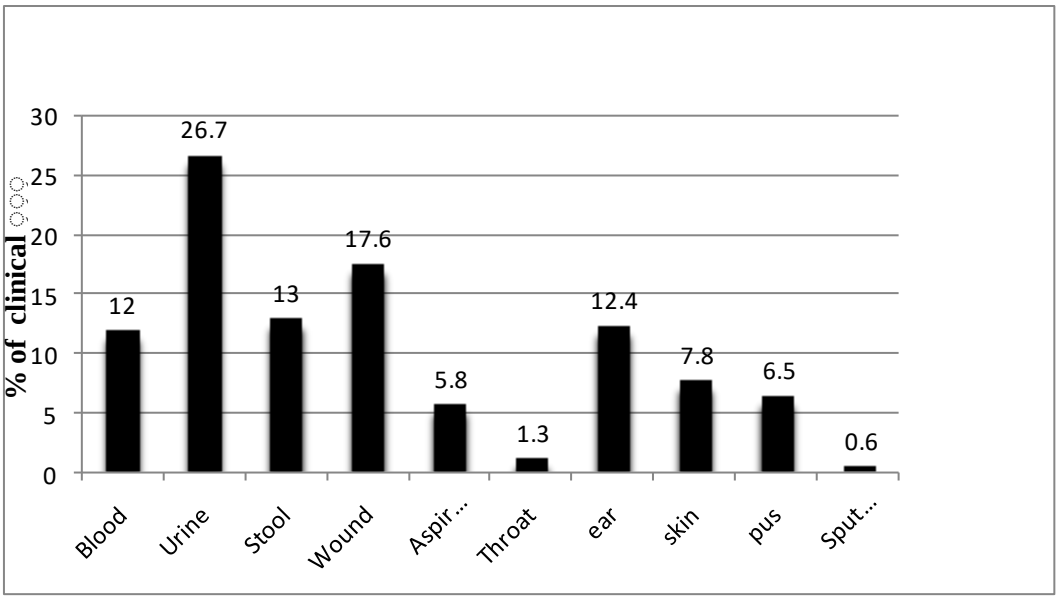

Figure 1: Percentages of clinical samples from which Enterobacteria were isolated.