Antimicrobial susceptibility test of microorganisms isolated from sputum culture of leukaemic patients with lower respiratory tract infections

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Summary

Background : *The development of respiratory infection indicates either a defect in host defenses, exposure to a particularly virulent microorganism, or an overwhelming inoculum, as infectious agents gain entry to the lower respiratory tract through aspiration of upper airway resident flora. Better prognosis of patients with leukaemia over the last decade is at least partly due to the possibility of administering more intensive chemotherapy and to the successful introduction a wider array of antimicrobials.*

Aim : *To evaluate the antimicrobial susceptibility of the isolates of both leukaemic and non-leukaemic patients with LRTIs.*

Methods : *The present study consisted of 50 adult leukaemic patients, 14 males and 36 females beside other 50 adult non-leukaemic patients, 25 males and 25 females were included, who were admitted to Baghdad Teaching Hospital, through the period from December 2003 through May 2004 with diagnosis of LRTIs. The antimicrobial susceptibility test was done upon the bacterial isolates according to Kirby-Bauer method.*

Results : *The most reliable antibiotics among leukaemic patients (acute myelogenous, acute lymphoblastic, chronic myeloid, chronic lymphocytic) according to antimicrobial susceptibility test, were in cosequence, ciprofloxacin, followed by cefotaxime, then gentamicin and equal influence by ceftriaxone, amikacin, cloxacillin followed by trimethoprime-sulphamethoxazole, ampicillin, augmentin, finally by erythromycin. On the other spectrum, the most reliable antibiotics among non-leukaemic patients were in consequence ciprofloxacin, followed by trimethoprime-sulphamethoxazole, cefotaxime, an equal effect by ampicillin, gentamicin, and ceftriaxone, followed by augmentin, also an equal effect by cloxacillin and amikacin, finally by erythromycin.*

Conclusion : *Antibiotic susceptibility test should be done for each bacterial isolate in order to prevent the development of progressive microbial resistance.*

Key words : *Antibiotic susceptibility test, lower respiratory tract infections, leukaemia.*

Introduction

Lower respiratory tract infections occur in both immunocompromised and nonimmuno-compromised patients. The classical example of the first group are patients affected by HIV or those who have undergone transplantation or antineoplastic chemotherapy such as leukaemic patients (1).

During the 1990s, a proportional increase in the incidence of Gram-positive bacteria as a cause of infections with a decrease in the occurrence of Gram-negative infections and a worrisome increase in lethal invasive fungal infections. The changes in the incidence of microorganisms of LRTIs of leukaemic patients are the result of alterations in the treatment of the haematologic malignancies, as well as adjusted anti-infective programmes (2,3). The possibility of a febrile episode in leukaemic patients being associated with drugs such as antibiotics, bleomycin, cytarabine or with the underlying disease should always be considered, but such association is usually quite apparent (4,5).

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Patients and methods :

Patients sputum specimens were cultivated within few minutes on blood, chocolate, MacConkey and two fresh Sabouraud's agar plates. Chocolate agar plate was incubated under CO2, using candle jar, other plates were incubated aerobically at 37°C for 18-24 hours and one Sabouraud's agar plate was incubated at room temperature for two weeks. Microorganisms from all specimens were identified according different microbiological and biochemical tests (6,7,8).

The inoculum was prepared from the primary yielded growth; the tops of 3-5 colonies of the same appearance were transfer to a tube of Brain-Heart infusion broth, incubate at 37°C for 3-5 hours (6).

The tube was compared with the turbidity standard (McFerland's solution) tube to adjust the density of the tested suspension to that of the standard. This adjustment is important to ensure that the resulting growth is confluent.

Mueller-Hinton agar plate was inoculated by dipping a sterile swab into the inoculum tube. The

excess inoculum was removed by pressing and rotating the swab against the tube walls above the liquid level. Then the swab was streaked all over the surface of Mueller-Hinton agar plate 3-4 times. The plate was rotated through an angle of 60° after each streak. Lastly the swab was passed over the plate edges, and left it to dry at room temperature for 10-15 minutes with its cover closed (9).

Then the chosen antibiotic discs were placed (Table 1) on the surface of the plate by pressing gently with a sterile forceps to ensure contact. The distance from the edges of the plate to the edge of the disc must be 15 mm, one disc placed in the center, five discs were applied for each test, incubate at 37°C overnight. Then the diameter of each inhibition zone (including the disc diameter) was measured by a ruler in mm on the under-surface of the plate. According to the measurements given in the table supplied by the manufacturer (which is belonged to Bioanalyse company, Turkey), the isolated organism was classified as susceptible, intermediate, and resistant to the antimicrobial agent.

Results :

Table (2) shows the AST for bacterial isolates from adult leukaemic patients. *Streptococcus pneumoniae (S.pneumoniae)* were hundred percent susceptible to cefotaxime (17/17), followed by ciprofloxacin (15/17), augmentin plus trimethoprime- sulphamethoxazole (14/17). Equal effect observed by ampicillin and erythromycin (13/17). Similar results were observed with amikacin, ceftriaxone (12/17). The susceptibility to gentamicin, cloxacillin were also equal (11/17).

The six isolates of *Staphylococcus aureus (S.aureus)* were hundred percent susceptible to cloxacillin, followed by ciprofloxacin (5/6). Equal numbers (4/6) were observed to each of ampicillin, augmentin, trimethoprime-sulphamethoxazole, cefotaxime, and ceftriaxone. Finally three isolates of *S.aureus* were susceptible to erythromycin, gentamicin, and amikacin respectively.

Moraxella catarrhalis (M.catarrhalis) was highly susceptible to ciprofloxacin (17/18), followed by cefotaxime and gentamicin (14/18). Obvious susceptibility were observed to cloxacillin, plus ceftriaxone (13/18), as well as amikacin (12/18). While (10/18) were susceptible to ampicillin, trimethoprime-sulphamethoxazole. The least susceptibility was to augmentin (7/18). The family *Enterobacteriaceae (Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca)* were nearly equal in their susceptibility, the best results were observed with gentamicin, ciprofloxacin, cefotaxime, ceftriaxone, followed by amikacin, trimethoprime-sulphamethoxazole. Less response was recorded to ampicillin, augmentin, and cloxacillin, while no role of erythromycin has been observed.

Pseudomonas aeruginosa (P.aeruginosa) was susceptible to gentamicin, amikacin, less susceptible towards trimethoprime-sulphamethoxazole, ciprofloxacin, cefotaxime, and ceftriaxone consecutively. The organism was resistant to ampicillin, augmentin, cloxacillin, erythromycin.

In the present work regarding the non-leukaemic patients, Table (3) shows that *S.pneumoniae* was exclusively susceptible to ampicillin and ciprofloxacin (20/22), followed by high susceptibility to trimethoprime-sulphamethoxazole (19/22), and cefotaxime (18/22).

An equal response of *S.pneumoniae* isolates were seen against augmentin, ceftriaxone (16/22). The influence of erythromycin, and cloxacillin upon the organism was (13/22), and (12/22) respectively. In addition, an equal influences was seen by gentamicin and amikacin (7/22).

It is obvious that the four isolates of *S.aureus* were sensitive to ciprofloxacin (4/4), followed by a good susceptibility to cloxacillin, trimethoprime-sulphamethoxazole, cefotaxime (3/4). Equal response to ampicillin, augmentin, erythromycin, ceftriaxone (2/4). While amikacin gave the least susceptibility (1/4).

Moraxella catarrhalis gain the big share as a causative agent among non-leukaemic patients (18 isolates), the best susceptibility was observed by ciprofloxacin (16/18), followed by gentamicin (15/18), amikacin (13/18), an equal influence by augmentin, cloxacillin, trimethoprime-sulphamethoxazole, and cefotaxime (12/18). Ten isolates were susceptible to ceftriaxone, the least influence was seen by ampicillin (6/18), erythromycin (3/18).

According to some members of family *Enterobacteriaceae*, the susceptibility to antibiotics were near to that expressed by the isolates of leukaemic patients group. The best results gained by amikacin, ciprofloxacin, followed by gentamicin, trimethoprime-sulphamethoxazole, cefotaxime in the first line, ampicillin, cloxacillin, ceftriaxone in the second line, augmentin plus erythromycin in the last line. The clear susceptibility of *P.aeruginosa* to gentamicin, and amikacin were obvious (3/3), then to cefotaxime, and ceftriaxone (2/3), no influence was seen by the rest antibiotics.

Table 1- Antimicrobial susceptibility discs employed in the current :

Antimicrobial agent	Code	Potency*
Ampicillin	AM	10mcg
Augmentin(Amoxicillin&clavulanic acid)	AMC	20mcg/10mcg
Cloxacillin	CX	1mcg
Erythromycin	E	15mcg
Gentamicin	GM	10mcg
Amikacin	AK	30mcg
Trimethoprime-sulphamethoxazole	SXT	1.25mcg/23.75mcg
Ciprofloxacin	CIP	5mcg
Cefotaxime	CTX	30mcg
Ceftriaxone	CRO	30mcg

* mcg: represent microgram.

Table 2- Antimicrobial susceptibility test of microorganisms isolated from sputum culture of leukaemic patients with lower respiratory tract infections.

Microorganisms	No. of susceptible isolates to antibiotics										
	No. of isolates	AM	AMC	CX	E	GM	AK	SXT	CIP	CTX	CRO
S. Pneumoniae	17	13	14	11	13	11	12	14	15	17	12
S. aureus	6	4	4	6	3	3	3	4	5	4	4
M. catarrhalis	18	10	7	13	4	14	12	10	17	14	13
E. coli	2	1	1	1	0	2	1	1	2	2	2
K. pneumoniae	1	1	1	1	0	1	1	0	1	1	1
K. oxytoca	3	1	1	2	0	3	3	1	2	2	1
P. aeruginosa	2	0	0	0	0	2	2	1	1	1	1

Table 3- Antimicrobial susceptibility test of microorganisms isolated from sputum culture of non - leukaemic Patients with lower respiratory tract infections.

Microorganisms	No. of susceptible isolates to antibiotics										
	No. of isolates	AM	AMC	CX	E	GM	AK	SXT	CIP	CTX	CRO
S. Pneumoniae	22	20	16	12	13	7	7	19	20	18	16
S. aureus	4	2	2	3	2	2	1	3	4	3	2
M. catarrhalis	18	6	12	12	3	15	13	12	16	12	10
E. coli	6	3	2	3	1	4	5	4	5	4	3
K. pneumoniae	4	4	2	2	0	4	3	4	4	3	2
K. oxytoca	1	1	0	1	0	1	1	1	1	1	1
P. aeruginosa	3	0	0	0	0	3	3	1	1	2	2

Discussion :

The antibiotics treatment which is recommended on the basis of the results of Gram stained sputum specimens differed significantly from the antibiotics treatment that had been described by the physician without performing a microscopic examination to the sputum specimens obtained from respiratory infection cases (10). Bacterial LRTIs are among the common health disorders requiring medical care and are associated with substantial morbidity, mortality, direct and indirect costs, antibiotics therapy should be guided by *invitro* AST (11,12).

It is of great interest to isolate and identify the causative agents incriminated with respiratory infections among adult immunocompetent patients whom complaining from LRTIs and chosen the proper antibiotics which act upon them, then compare it to another causative agents group incriminated with immunocompromised patients with a proper AST for them (13).

Interestingly it was found in the present study that most isolates of *S.pneumoniae* were susceptible to ciprofloxacin. This result discrepant from other study result (14), which revealed that in immunocompromised leukaemic patients with suspected or proven pneumococcal respiratory infections, it may be prudent not to use ciprofloxacin monotherapy empirically when the patient has a history of ciprofloxacin therapy in at least the past 4 months due to development of drug resistance.

It was of valid importance to notice that trimethoprime-sulphamethoxazole (SXT) in leukaemic patients, was effective against *M.catarrhalis*, *S.aureus*, *Enterobacteriaceae*, and *P.aeruginosa*. The latter is a major problem as a multiresistant nosocomial pathogen, especially in immunocompromised leukaemic patients in the hospitals. The present study further suggests that the leukaemic patients are more susceptible to acquiring infections due to multi resistant *P.aeruginosa* than other types of patients and the common site of infections were the respiratory tract. This is in agreement with studies results reported (15,16).

The current study results concerning antibiotics action upon microorganisms isolated from the non-leukaemic patients revealed that, most of the Gram-negative bacterial isolates from respiratory infections are varying in their resistance to the antibiotics. This result in consistence with other researches (13,17,18,19). The effectiveness of them were in consequence ciprofloxacin as the first drug of choice similar to the leukaemic group, followed by trimethoprime-sulphamethoxazole, then cefotaxime, an equal effect by ampicillin, gentamicin, and ceftriaxone, followed by augmentin, also an equal effect by cloxacillin and amikacin, finally the least effect was seen by erythromycin.

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