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Evaluation of Resistance in Clinical Isolates of E. coli, S. aureus, and P. aeruginosa against β-lactam Antibiotics and Gentamicin.

Rukhsana Firdous *, Safia Ahmed**, Shabbir Ahmad Chaudhary***, Ahsan Ahmad****, Naeem Akhtar*

*Department of Pathology,Rawalpindi Medical College, Rawalpindi;**Department of Microbiology,Quaid-i-Azam University, Islamabad,;***Department of Anatomy, Alnafees Medical College, Islamabad**** Department of Medicine, Fauji Foundation Hospital, Rawalpindi.

Abstract

Background: **To study the susceptibility pattern of β-lactam antibiotics and gentamicin in pathogenic clinical isolates with reference to the site of infection.**

Methods: **In this descriptive study three prevalent clinical isolates were selected i.e., Escherichia coli, (n = 203) Staphylococcus aureus (n = 194) and Pseudomonas aeruginosa (n = 106). These were isolated from urine, pus, high vaginal swabs, and a miscellaneous group, comprised of blood and fluid. The antibiotics included in the study were from β-lactam group and gentamicin.**

Results: **E. coli was mostly isolated from urine (n = 124), while S. aureus and P. aeruginosa were mostly isolated from pus samples. Among β-lactams the activity of imipenem was highly sensitive against all isolates as only 2.66 % of E. coli, 5.8% of S. aureus and none of P. aeruginosa isolates were resistant to imipenem. Highest resistance in these isolates was against ampicillin, where S. aureus was rather sensitive to this drug (44%) as compared to E. coli (15% sensitivity) and P. aeruginosa (13%). Staphylococcus aureus isolates were more sensitive to amoxicillin/clavulanate (75%) as compared to E. coli (58%) & P. aeruginosa (41%). Pipracillin/tazobactam and 3rd generation cephalosporins were also comparatively more sensitive (10-20% resistance). Staphylococcus aureus isolates showed 76% sensitivity against gentamicin while 72% of P. aeruginosa isolates and 65% of E. coli isolates were sensitive to this drug.**

Conclusion: **Regular evaluation of antibiotic sensitivity can assist in devising empiric therapy**

Introduction

Intrinsic resistance to an antibiotic can best be described as resistance of an entire species to an antibiotic, based on inherent (and inherited) characteristics requiring no genetic alteration. This is usually due to the absence of a target for the action of a given antibiotic or the inability of a specific drug to reach its target. For example, mycoplasmas are always resistant to β-lactam antibiotics since they lack peptidoglycan (which the β-lactams act upon). Similarly, the outer membrane of gram negative cells can prevent an antibiotic from reaching its target. For example, Pseudomonas aeruginosa exhibits high intrinsic resistance to many antibiotics due to its drug efflux pumps and restricted outer membrane permeability. The major mechanisms of acquired resistance are the ability of the microorganisms to destroy or modify the drug, alter the drug target, reduce uptake or increase efflux of the drug, and replace the metabolic step targeted by the drug. [1]

The mechanism of β-lactam resistance is via the action of the β-lactamases. These enzymes catalyze hydrolysis of the β-lactam ring and, thereby, inactivate these antibiotics. There are also some bacteria that possess altered penicillin binding proteins that result in reduced penicillin binding. Several compounds, such as clavulanic acid, have been discovered that have the ability to bind irreversibly to β -lactamases and, thereby, inhibit their action. Combinations of these compounds with β-lactam drugs have been very successful in treatment of disease. [2]

Materials and Methods

Samples were collected from both out patient Department (OPD) and hospitalized patients, at department of Pathology, Benazir Bhutto Hospital, Rawalpindi. The sensitivity pattern of clinically significant pathogenic bacteria, isolated from the samples was determined against commonly used β-lactam antibiotics and gentamicin, by disk diffusion method. Five hundred and three bacterial isolates which included Escherichia coli (n= 203) Staphylococcus aureus (n= 194) and Pseudomonas aeruginosa (n= 106) were chosen after due identification. 242 isolates were from pus samples, 189 isolates were from urine samples, and 45 were from

high vaginal swabs (HVS). All other samples were low in number, and altogether the miscellaneous group comprised of samples of blood and fluid.For Morphological Identification colony size, shape, diameter and growth characteristics (abundant, thin, pigment production) and gram stain were noted. Where required biochemical tests were performed .

Results

Resistance of E. coli isolates to β-lactam drugs and gentamicin: Third generation cephalosporins were quite effective for E. coli isolates and the edge was taken by the cefoperazone (CFP) . Pipracillin/ tazobactam (TZP) was also found to be quite effective as it showed resistance to 15.155 isolates. The most effective was imipenem. (Table 1)

Resistance in S-aureus isolates to β-lactam and gentamicin: Staphylococcus aureus isolates showed resistance to imipenem (5.8%). In 3rd generation cephalosporin, cefoperazone was found more effective (Table 1;Fig 1)

Resistance of P.aeruginosa to β-lactam antibiotics and gentamicin: Imipenem was found 100% sensitive. Efficacy of TZP was remarkable(Table 3&4).

Table 1:Susceptibility pattern of E. coli isolates to beta lactam antibiotics.

	Number of resistant organisms to antibiotics (%)									
Sample	AMP	AMC	V	CTX	IMP	CEC	CAZ	CFP	TZP	GEN
Urine	62(91.17)	43(44.32)	70(57.85)	31(25.4)	2(2.15)	26(61.9)	26(23.21)	18(18.75)	15(15.15)	47(39.83)
N=124	N=68	N=97	N=121	N=122	N=93	N=42	N=112	N=96	N=99	N=118
Pus	22(73.33)	16(39)	26(59)	7(15.9)	1(3.57)	7(46.66)	7(17.5)	3(9.38)	3(9.09)	13(28.88)
N=45	N=30	N=41	N=44	N=44	N=28	N=15	N=40	N=32	N=33	N=45
H.V.S	9(81.81)	7(35)	10(47.62)	1(4.45)	1(4.76)	0	4(19.0)	2(11.11)	0 N=14	4(23.5)
N=21	N=11	N=20	N=21	N=22	N=21	N=5	N=21	N=18		N=17
Mis	4(80)	5(45.45)	5(41.66)	1(8.33)	0	0	3(27.27)	1(11.11)	0	5(38.46)
N-13	N=5	N=11	N=12	N=12	N=8	N=0	N=11	N=9	N=10	N=13
Total N=203	97(85)	71(42)	111(56)	40(20.1)	4(2.66)	33(53.3)	40(21.73)	24(15.48)	18(11.54)	69(35.75) N=193

AMP,ampicillin; AMC,amoxicillin/clavulanate; CTX,cefotaxime; IMP imipenem ;MET,methicillin; CEC,cefaclor; CAZ,ceftazidime; CFP,cefoperazone; TZP,pipracillin/tazobactam and GEN, Gentamicin

Table-2:Susceptibility pattern of S. aureus isolates to beta lactam antibiotics

	Number of resistant organisms to antibiotics (%)									
	AMP	**AMC**	**V**	**CTX**	**IMP**	**MET**	**CEC**	**CAZ**	**CFP**	**TZP**
H.V.S	3(20) N=15	1(5.8) N=17	4(23.52) N=17	3(18.7) N=16	0(0) N=13	3(50) N=6	1(25) N=4	2(15.38) N=13	2(16.66) N=12	0(0) N=13
Pus	74(64.9) N=114	35(30.43) N=115	45(34.6) N=130	18(14.17) N=127	7(6.48) N=108	7(12) N=58	18(50) N=36	17(15.88) N=107	9(8.7) N=103	12(11.53) N=104
Misc	2(28.57) N=7	2(22.22) N=9	2(22.22) N=9	2(22.22) N=9	0(0) N=7	1(50) N=2	2(66.66) N=3	1(12.5) N=8	0(0) N=5	0(0) N=5
Urine	17(50) N=34	7(21.2) N=33	15(44.1) N=34	5(15.62) N=32	2(7.4) N=27	7(46.6) N=15	6(50) N=12	6(23) N=26	4(15.3) N=26	5(20) N=25
Total	96(56.47)	45(25.8)	66(35.10)	28(15.21)	9(5.8)	18(22.22)	27(49)	26(16.8)	15(10.2)	17(11.5)

AMP,ampicillin;AMC, amoxicillin/clavulanate; V,cephradine; CTX, cephotaxime; IMP, imipenem; MET, methicillin; CEC,cefaclor; CAZ,ceftazidime;CFP, cefoperazone; TZP, pipracillin/tazobactam

Table-3:Susceptibility pattern of P.aeruginosa isolates to beta lactam antibiotics

Number of resistant organisms to antibiotics (%)

Sample	AMP	AMC	V	CTX	IMP	CEC	CAZ	CFP	TZP
Urine	15(83.3)	13(56.5)	19(63.3)	8(26.6)	0	8(80)	3(11.5)	4(16)	3(11.5)
	N=18	N=24	N=28	N=29	N=26	N=10	N=26	N=25	N=26
Pus	34(91.8)	35(66)	48(73.8)	18(27.69)	0	21(91.3)	7(12)	6(12.5)	5(10.2)
	N=37	N=53	N=64	N=65	N=46	N=23	N=60	N=48	N=49
H.V.S	5(71.4)	3(42.8)	4(57.1)	0	0	0	0	0	0
	N=7	N=7	N=7	N=7	N=0	N=7	N=7	N=5	N=6
Misc.	1(100)	1(100)	3(75)	2(50)	0	0	0	1(25)	0
	N=1	N=1	N=4	N=4	N=3	N=2	N=3	N=4	N=3
Total	55(87.3)	52(59)	74(71.8)	60(43.1)	0	29(82.8)	10(10.6)	11(13.4)	8(9.52)

AMP,ampicillin;AMC, amoxicillin/clavulanate;V,cephradine;CTX, cephotaxime; IMP,Imipenem; MET,methicillin; CEC,cefaclor;CAZ,ceftazidime;CFP,cefoperazone;TZP, pipracillin/tazobactam

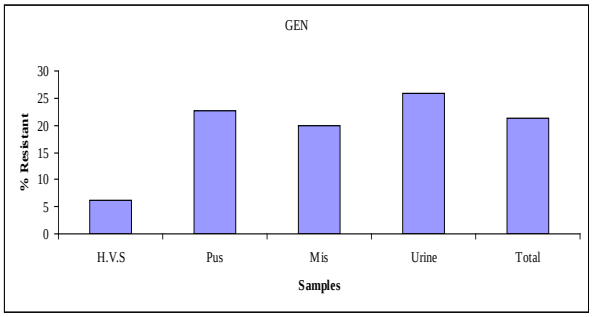

Fig-1:Resistance of S.aureus to gentamicin

Table-4:Resistance of P. aeruginosa to gentamicin

Sample	Gentamicin (% resistance)
H.V.S (n=7)	0
Pus (n=62)	19(30.6)
Miscellaneous(n=4)	0
Urine (n=33)	10(30.3)
Total=105	30(28.57)

Discussion

In present study resistance to beta lactam in E. coli isolates showed high resistance against ampicillin, amoxicillin-clavulanate, cephradine, and cefaclor(40%) Results are consistent with prior published studies revealing increasing resistance to both ampicillin and cephradine, in this part of world. [3] Highly sensitive antibiotic was found to be imipenem, to which only 2.66% isolates gave resistance.

In a study done by Whenzel during 2000 and 2001 in four European countries, Canada and United States, in hospitalized patients. E. coli susceptibility to cefotaxime was 97% or greater in each country. Sensitivity to ampicillin was from 39-58%. Lowest sensitivity was from Spain while the highest sensitivity was from Germany. Sensitivity to amoxicillin-clavulanate was highest in Spain i.e 85.8%. Data from other countries ranged from 69.9 to 81.2%. Cefotaxime showed sensitivity ranging from 97.1% to 99.6%. Ceftazidime showed results close to cefotaxime i e, 97-99.1%. Imipenem remained 100% sensitive in all countries. Piperacillin-tazobactam was sensitive to 95-97% isolates throughout these countries. [4]

During a survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections, Kahlmeter reported resistance from 17 countries in uncomplicated community acquired urinary tract infections in women less than 65 years of age. Resistance was most common to ampicillin (29.8%). Resistance in E. coli to co-amoxiclav, was 21.3%. [5] The resistance to ampicillin and co-amoxiclav, is lower than our study, because their study included only uncomplicated community acquired urinary tract infections. Similarly in a study Goldstein, reported in community acquired urinary tract infections in France and showed higher rates of susceptibility in Escherichia coli, to amoxicillin-clavulanic acid (63.3%), cephalothin (66.8%) cefotaxime (99.8%), ceftazidime 99%. [6] In the present study the sensitivity to amoxicillin-clavulanic acid was 56%, slightly less than Goldstein, as our study also included hospitalized patients.

The present study revealed that in case of S. aureus isolates from pus, ampicillin and cefaclor

showed comparatively high resistance (56% and 50%) followed by Cephradine (34.6%) and then amoxycillin-clavulanic acid and methicillin (26% and 25.8%). Ampicillin resistance 56 % in our study is comparable to Sattar. [7] There are reports from many countries, of isolates with reduced susceptibility to vancomycin and teicoplanin frequently associated with a significant thickening of the cell wall. [8] Methicillin resistant S. aureus which was not the target of our study, showed 12% pus isolates and 46.6% uropathogens were resistant which is comparable to other reports. [9] Pfaller, also reports a high incidence of MRSA (28.3%) among urinary tract infection isolates and this was lowest among isolates associated with skin and soft tissue infections (22.4%).[10] These differences might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure.

Conclusions

1.E. coli is the most prevalent bacteria in the aetiology of urinary tract infections.

2.For empiric therapy of urinary tract infections, Pipracillin-Tazobactam can be prescribed.

3.Among third generation cephalosporins, Cefoperazone has an edge over Ceftazidime and Cefotaxime.

4.S.aureus and P.aeruginosa are the prevalent pathogens in skin and soft tissue infections. In these cases Pipracillin-Tazobactam and Cefoperazone could be prescribed empirically. Ceftazidime has an edge when this infection is hospital acquired, due to the higher isolation of P.aeruginosa in nosocomial infections globally.

5.Imipenem , with a high efficacy against these three most common pathogens, can be prescribed in problematic infections.

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