

Original Article

Antibiotic sensitivity pattern of *Klebsiella* species in burn wounds at Bai Jerbai Wadia hospital for children, Mumbai, India-a 21 year study

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Abstract: In our study, we attempt to look at the sensitivity pattern of the most commonly found microorganism in the wound swab of pediatric burn patients at our hospital, 'Klebsiella', to a number of commonly used antibiotics and to determine whether there has been a change in the sensitivities in the 21 year period between 1994 to 2014. The sensitivity was tested in vitro and antibiotics were administered to patients based on laboratory findings. Klebsiella shows a significant change in sensitivity pattern over last 21 years. The sensitivity of this organisms to various antibiotics tested has reduced over time. The organism out plays most of the antibiotics that it is subjected to in vitro. Our efforts should now be channelized towards limiting the use of antibiotics or a proper antibiotic policy which exercises control of irrelevant and excessive use of antibiotics.

Keywords: Klebsiella, antibiotics, resistance

Introduction

The control of infections is seriously threatened by the steady increase in the number of microorganisms that show resistance to anti microbial agents. Resistant infections adversely affect mortality, treatment costs, disease spread and duration of illness.

In this study, we attempt to look at the sensitivity pattern of the most commonly found microorganism in the wound swab of pediatric burn patients, 'Klebsiella', to a number of commonly used antibiotics and to determine whether there has been a change in the sensitivities in the 21 year period between 1994 to 2014.

Materials and methods

Between 1994 and 2014, 2323 pediatric patients in the age group between 1 month and 15 years were admitted to our Burns unit, an

exclusive pediatric unit in the city. Microbiological samples were collected (11493 swabs were processed) and their bacteriology and antibiotic sensitivities recorded. 17507 isolates were detected.

Wound treatment

Closed dressings using silver sulphadiazine ointment were used in all patients without exception. The burn wounds were washed daily to remove necrotic tissue and the remnants of the previous day's ointment.

Procedure for wound sampling

Microbial colonization of all wounds was studied from the time of admission to discharge. On admission, the sampling procedure included swabs that were taken from clinically deep areas of the burn wound prior to any cleansing. Swabs were taken twice weekly. The bandages

Evolving resistance of klebsiella species

Table 1. Antibiotic sensitivity-Penicillins & Carbapenems

Year	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	Total		
Total Isolates	862	1927	1249	626	592	539	307			
Antibiotics	%	%	%	%	%	%	%	Tested	NO	%
Penicillin	2.9	5.6	NT	NT	NT	NT	NT	2789	131	4.7
Ampicillin	2.8	5.5	5.3	3.2	2.3	1.0	7.4	6102	258	4.2
Cloxacillin	1.9	1.2	2.7	1.1	2.3	1.00	7.4	6102	154	2.5
Carbenicillin	3.2	11.2	6.8	9.3	NT	NT	NT	4664	356	7.6
Piperacillin	10.1	24.7	39.6	15.6	9.2	5.8	NT	5795	1013	17.5
Ticarcillin	10.1	27.5	NT	NT	NT	NT	NT	2789	399	14.3
Imipenem	40.5	68.5	71.3	44.6	60.2	81.4	89.57	5651	3828	67.7
Amoxicillin + Clavulanicacid	NA	NA	NA	6.1	13.2	5.3	16.9	2064	214	10.4
Piperacillin + Tazobactam	NA	NA	NA	78.2	68.9	78.3	64.2	2064	1493	72.4
Ticarcillin + Clavulanicacid	NA	NA	NA	32.3	29.0	6.7	20.5	2064	456	22.1
Meropenem	NA	NA	NA	76.6	65.3	57.0	84.4	2064	1461	70.8
Ertapenem	NA	NA	NA	NA	NA	30.2	59.6	499	260	52.3
Faropenem	NA	NA	NA	NA	NA	15.0	50.1	499	206	41.3
Doripenem	NA	NA	NA	NA	NA	29.6	61.8	499	268	53.8
Aztreonam	NA	NA	NA	NA	NA	24.4	38.4	846	265	31.4

Table 2. Antibiotic sensitivity-Cephalosporins

Year	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	Total		
Total Isolates	862	1927	1249	626	592	539	307			
Antibiotics	%	%	%	%	%	%	%	Tested for	NO	%
Cephalexin	7.6	21.6	18.0	3.0	5.0	NT	NT	5256	578	11.0
Cephazolin	23.3	28.3	21.3	17.3	3.0	0.5	NT	5795	904	15.6
Cefuroxime	52.6	49.3	45.6	5.6	4.2	8.7	11.7	6102	1830	30.2
CephadroxyI	38.0	20.6	17.6	5.6	3.3	NT	NT	5256	893	17.0
Cefotaxim	57.3	50.0	46.3	17.0	11.3	8.1	24.4	6102	1832	30.7
Ceftazidime	29.6	30.3	52.6	21.0	19.5	6.6	NT	5795	1535	26.5
Ceftriaxone	52.6	42.0	53.0	15.3	16.0	10.0	NT	5795	1831	31.6
Ceftizoxime	67.3	50.6	65.3	28.0	24.9	25.4	43.6	6102	2648	43.4
Cefoperazone	68.0	43.3	46.0	25.6	10.6	2.5	NT	5795	1889	32.6
Cefaclor	NA	NA	20.3	5.3	NT	NT	NT	1875	243	13.0
Cefixime	NA	NA	50.6	5.0	4.5	5.9	NT	3006	480	16.5
Cefpirome	NA	NA	68.6	28.0	12.6	4.4	NT	3006	856	28.5
Cefepime	NA	NA	NA	35.00	14.80	7.40	NT	1757	339	19.3
Cefoperazone + sulbactam	NA	NA	75.6	86.6	81.1	77.5	69.0	3313	2584	78.0
Cefotaxim + sulbactam	NA	NA	NA	NA	64.3	56.2	NT	1131	678	60.0
Ceftriaxone + sulbactam	NA	NA	NA	NA	55.3	49.7	60.2	1438	790	55.0
Cefixime + Clav	NA	NA	NA	NA	NA	NA	25.7	307	78	25.7
Cefoperazone + Tazobactam	NA	NA	NA	NA	NA	NA	48.2	307	147	48.2
Ceftazidime + Tazobactam	NA	NA	NA	NA	NA	NA	55.3	307	169	55.3
Ceftriaxone + Tazobactam	NA	NA	NA	NA	NA	NA	57.6	307	176	57.6
Cefepime + Tazobactam	NA	NA	NA	NA	NA	NA	84.4	307	259	84.4

were removed and the wounds are washed. The wounds were swabbed and cultured as follows: A sterile cotton swab is moistened with sterile normal saline. This swab is rubbed onto the

burn wound surface. Swabs are taken from areas which appear deep, areas with discharge or thick eschar. The swabs are then sent immediately for culture.

Evolving resistance of klebsiella species

Table 3. Antibiotic sensitivity-Aminoglycoside

Year	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	Total		
Total Isolates	862	1927	1249	626	592	539	307			
Antibiotics	%	%	%	%	%	%	%	Tested for	NO	%
Streptomycin	48.3	33.3	NT	NT	NT	NT	NT	2789	1137	40.8
Gentamycin	18.3	31.3	26	13.5	21.6	23.3	37.1	6102	1494	24.5
Tobramycin	24.3	28.6	38.6	19.0	35.2	30.8	58.6	6102	2038	33.4
Amikacin	77.3	59.6	79.6	51.0	62.8	59.1	61.5	6102	3923	64.3
Netilmycin	38.6	42.0	79.3	48.0	55.1	61.4	63.5	6102	3382	55.4

Table 4. Antibiotic sensitivity-Fluroquinolones

Year	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	Total		
Total Isolates	862	1927	1249	626	592	539	307			
Antibiotics	%	%	%	%	%	%	%	Tested for	NO	%
Ofloxacin	66.0	59.0	59.0	36.0	32.6	25.4	NT	5795	2685	46.3
Pefloxacin	47.3	54.0	50.6	27.0	19.3	12.0	NT	5795	2028	35.0
Norfloxacin	52.3	49.3	43.3	23.0	24.5	21.8	NT	5795	2057	35.5
Ciprofloxacin	61.0	59.0	56.3	32.0	49.2	55.4	44.3	6102	3120	51.1
Sparfloxacin	NA	NA	74.0	51.6	62.9	57.3	81.7	3313	2173	65.6
Lomefloxacin	NA	NA	67.3	43.3	51.3	26.9	45.9	3313	1550	46.8
Gatifloxacin	NA	NA	NA	86	76.5	78.2	NT	1757	1411	80.3
Levofloxacin	NA	NA	NA	NA	NA	NA	90.8	307	276	90.8

Table 5. Antibiotic sensitivity-Macrolides

Year	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	Total		
Total Isolates	862	1927	1249	626	592	539	307			
Antibiotics	%	%	%	%	%	%	%	Tested for	NO	%
Erythromycin	12.6	27.3	17.0	3.2	9.5	10.9	14	6102	828	13.6
Azithromycin	NA	NA	46.6	42.6	49.6	53	66.8	3313	1729	52.2
Roxithromycin	NA	NA	13.3	6.3	9.4	6.6	8.14	3313	649	19.6
Clarithromycin	NA	NA	23.3	14.0	11.6	5.38	10.0	3313	437	13.2

Microbiology

The swabs are transported to the laboratory for processing immediately. They are streaked onto a differential medium (e.g.; Mac Conkey agar) and an enriched medium (e.g. blood agar). Isolation is carried out by the conventional T-method using sterile nichrome loop. These plates are incubated at 37°C for 16-18 h. The basic aim was to isolate the organisms predominant on the burn wound and determine their sensitivity to various antibiotics for clinical purposes.

Antibiotic sensitivity of isolates obtained from the burn wound was carried out by Agar disc diffusion method (Kirby Bauer method) [1]. Sterile commercially available filter paper discs,

onto which a definite amount of antibiotic has been absorbed, are used. Since the antibiotic in the disc tends to diffuse more onto the surface of the agar than into the deeper layers, the plate is surface spread with the organisms. A broth culture of the isolate is prepared using sterile peptone water comparable to 0.5 McFarland's turbidity standard (i.e. 1×10^7 to 1×10^8 organisms/ml). Approximately 0.2 ml of this broth culture is surface spread onto sterile Mueller Hilton agar so as to get a matt growth. Sterile antibiotic discs are equidistantly placed on these plates and gently pressed onto the medium with the help of sterile forceps to ensure complete contact with the agar surface. The plates are incubated at 37°C for 16 to 18 h. Zone of inhibition was measured in millimeters and sensitivity reported.

Evolving resistance of klebsiella species

Table 6. Antibiotic sensitivity-Other antibiotics

Year	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	Total		
Total Isolates	862	1927	1249	626	592	539	307			
Antibiotics	%	%	%	%	%	%	%	Tested for	NO	%
Colistin	56.3	18	NT	NT	50.3	46.5	61.5	4227	1454	34.4
Co-trimoxazole	7.6	19.6	11.6	13.3	16.4	6.3	21.1	6102	823	13.5
Tetracycline	20.3	18.6	27.0	15.3	30.9	28.0	51.1	6102	1659	27.2
Chloramphenicol	37.3	30.3	40.3	31.6	41.7	23.7	72.3	6102	2404	39.4
Metronidazole	3.26	0.0	0.0	0.0	0.0	0.0	0.0	6102	24	0.4
Clindamycin	NA	NA	40.0	11.3	15.3	1.1	1.0	3313	457	13.8
Spiramycin	NA	NA	13.6	4	NT	NT	NT	1875	168	9.0
Tigecycline	NA	NA	NA	NA	NA	NA	95.4	307	292	95.4

Abbreviations: NT-Not tested; NA-Not Available.

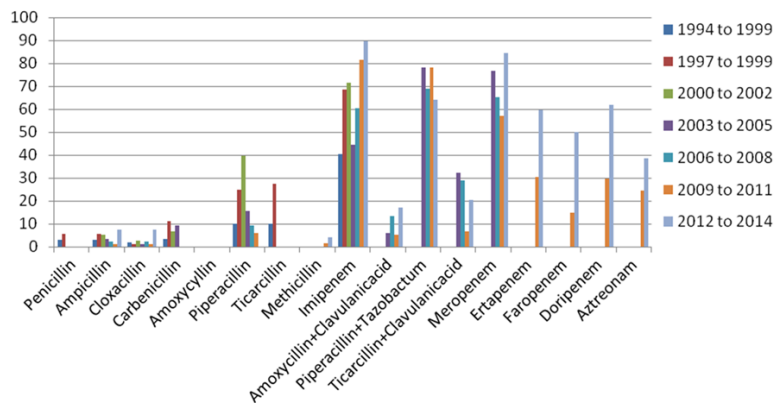


Figure 1. Graphical representation of antibiotic sensitivity (%) of Penicillin and Carbapenems.

Observations: (Tables 1-6)

Among 11493 microbiological samples which were taken during the study period, 17507 bacterial strains were found. The frequency of Klebsiella (6102) was found to be 33.9%. Broadly, the antibiotic sensitivities were as follows.

Klebsiella showed more than 50% sensitivity to the following antibiotics: Tigecyclin (95.4%); Levofloxacin (90.8%); Cefepime + Tazobactam (84.4%); Gatifloxacin (80.3%); Cefoperazone + Sulbactam (78%); Piperacillin and Tazobactam (72.38%); Meropenem (70.8%); Imipenem (67.74%); Amikacin (64.3%); Azithromycin (52.2%).

Results

Penicillins (Table 1, Figure 1)

Up to 2005, Piperacillin with tazobactam showed 78.2% sensitivity. This percentage is

coming off gradually (64% in 2014) as the use of Piperacillin and Tazobactam increases. The most prevalent resistance mechanism against beta lactams is the production of beta lactamases. While many beta lactamases are not capable of hydrolyzing cephalosporins, the ESBLs (extended spectrum beta lactamases) have this ability. They are commonly found in Klebsiella and other Enterobacteriaceae. Some of the in vitro resistance to Piperacillin-

Tazobactam may be explained by the production of ESBL [2].

Carbapenems (Table 1)

Meropenem demonstrates better sensitivity till 2005 (76.6%) but since 2006, this is also shows a dip. Newer carbapenems (Ertapenem, Doripenem, Faropenem, and Aztreonam) show lesser sensitivity than Imipenem (67.74%) and meropenem (70.8%).

Cephalosporins (Table 2, Figure 2)

The best sensitivities were recorded with Cefepime + Tazobactam (84.4%) followed by cefoperazone and sulbactam (78%) and cefotaxime and sulbactam (60%).

Most of the other cephalosporins had to be discontinued due to its diminishing sensitivity pattern.

Evolving resistance of klebsiella species

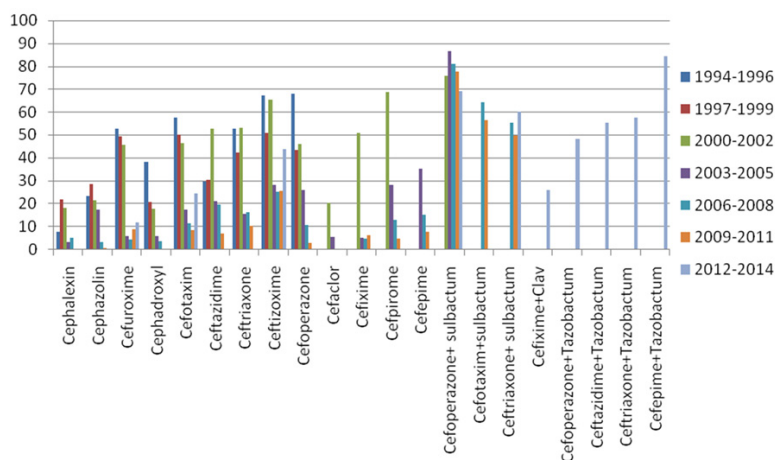


Figure 2. Graphical representation of antibiotic sensitivity (%) of Cephalosporins.

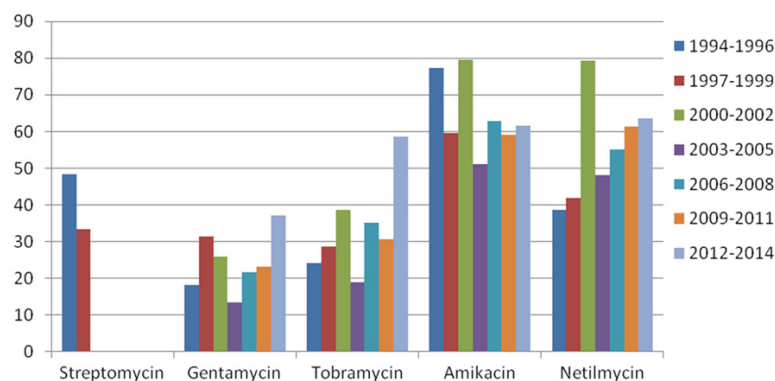


Figure 3. Graphical representation of antibiotic sensitivity (%) of Aminoglycosides.

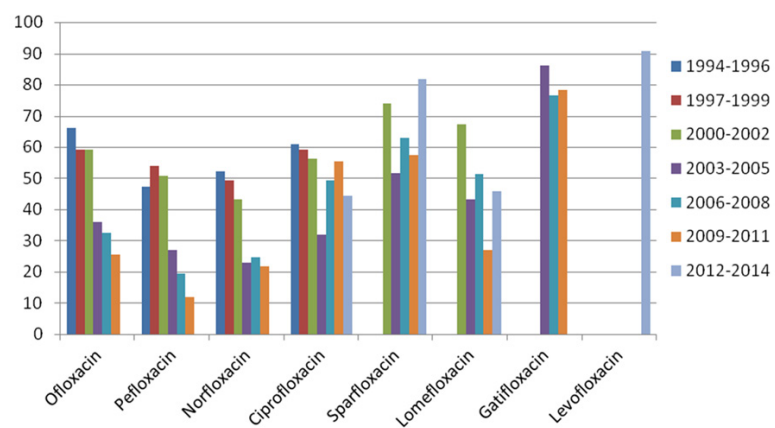


Figure 4. Graphical representation of antibiotic sensitivity (%) of Fluoroquinolones.

Aminoglycosides (Table 3, Figure 3)

Amikacin (64.3%) and Netilmycin (55.4%) have continued to prove efficacious in our series.

Gentamycin showed 24.5% sensitivity.

Fluoroquinolones (Table 4, Figure 4)

Levofloxacin emerged the forerunner with 90.8% sensitivity followed by Gatifloxacin (80.3%) and Sparfloxacin (65.6%). Ofloxacin, pefloxacin, norfloxacin showed a steady decline in the sensitivity pattern and hence were discontinued.

Macrolides (Table 5, Figure 5)

Azithromycin emerged numero uno with 52.2% of strains being sensitive to it. The others of this group have always been ineffective against klebsiella.

Other antibiotics (Table 6, Figure 6)

Tigecycline has shown 95.4% sensitivity in last 3 years. The sensitivity pattern of tigecycline will have to be watched closely over time.

Chloramphenicol has shown encouraging increase in sensitivity between 2012 to 2014.

Colistin has also maintained its sensitivity between 2006 to 2014.

Discussion

Thermal injury destroys protective skin barrier, allowing colonization of wound with micro-organism. The largest and most repeatedly encountered groups of microorganisms populating any burn wound environment are the

enteric bacteria which belong to the family Enterobacteriaceae. This family consists of 12 individual genera. Many of them were previously thought to be non pathogenic. They are often

Evolving resistance of klebsiella species

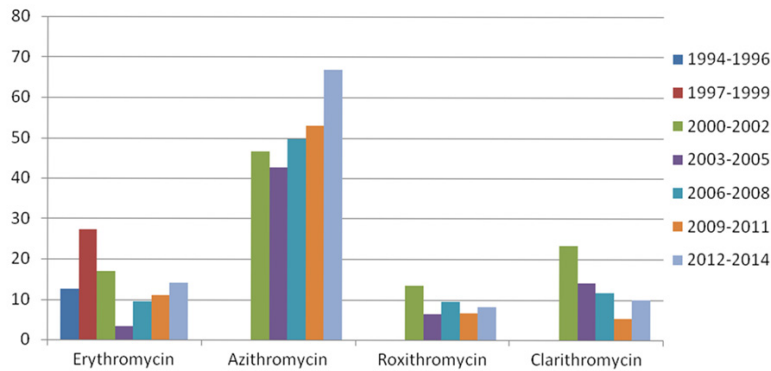


Figure 5. Graphical representation of antibiotic sensitivity (%) of Macrolids.

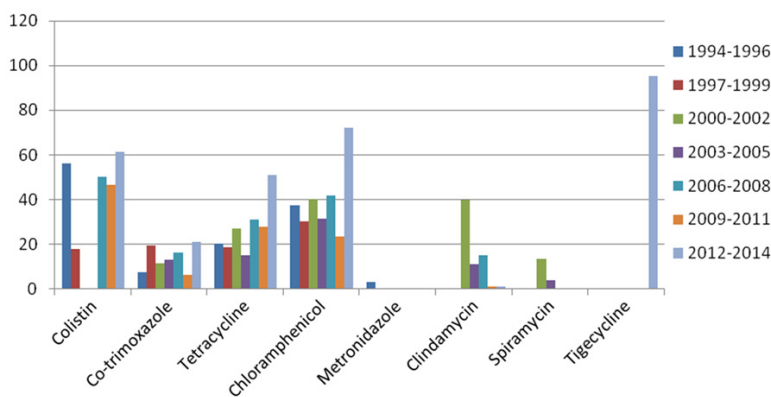


Figure 6. Graphical representation of antibiotic sensitivity (%) of other antibiotics.

present in the burn wound, originating either in the patient's bowels or from the burn wound environment. Their antibiotic susceptibility pattern varies widely [3].

In the literature we perused, it was surprising that was a paucity of data from Indian centers [4]. It was a revelation going against the conventional grain; Klebsiella was the dominant organism in our setup. Fadeyibi et al from Nigeria report that in their series, Pseudomonas and Proteus were predominant organisms [5]. Sarita et al found Staphylococcus to be most common organism while Klebsiella was most common gram negative organism isolated [6]. At Malawi, Klebsiella accounted for 3.5% of the isolates [6]. In Korea, Klebsiella was isolated in 53 out of 6550 isolates (0.8%) [8]. In the study done by Idomir et al the etiological spectrum of the burn wound infections was dominated by Gram positive cocci (65%) [9]. Mehta M at Chandigarh, India found Pseudomonas to be

commonest organism in their eight year study [10].

The genus Klebsiella consists of nonmotile, non spor-ing, non acid fast, and cap-sulated gram negative rods. They are widely distributed in nature, occurring both as commensals in intestines and as saprophytes in soil and water. It is the second most populous member of the aerobic bacterial flora of the human intestine. It has become an important cause of nosocomial infections, replacing even E.coli in some centers. They grow on ordinary media producing large, dome shaped and mucoid colonies of various degrees of stickiness. They are classified into three species- K. pneumoniae, K. ozaenae and K. rhinoscle-romatis [11].

As with any other organism, the antibiotic sensitivity pat-tern of Klebsiella too has undergone a sea change.

Arfaz and Twum-Danso from Saudi Arabia report that all the Klebsiella in their series were resistant to ampicillin, but 24% and 14% were resistant to Tobramycin and Gentamycin respectively and 12% to Piperacillin [12].

Beta lactam antibiotics have been prescribed to treat serious infections for over 60 years. They too have fallen victim to the menace of resistance. Beta lactamase enzyme pro-duction is the primary mode of resistance to beta lactam antibiotics. These enzymes are produced by virtually all gram negative bacte-ria, esp. Escherichia and Klebsiella. In the mid eighties, it became evident that a new type of beta lactamase was being produced which could hydrolyse extended spectrum cephalo-sporins (eg: third generation cephalosporins). These new beta lactamases have been termed Extended Spectrum Beta Lactamases (ESBL) [2]. They are easily transferable. Monobactams like aztreonam are also inactivated. Wide-

Evolving resistance of klebsiella species

spread use of aztreonam and cephalosporins are believed to be the major cause of mutations in the genes that have led to the emergence of ESBLs.

What then, is the best method to control outbreaks of ESBL producing *Klebsiella*? The cornerstone of any strategy, says Rice is to minimize administration of extended spectrum cephalosporins, especially Ceftazidime [13]. In most cases, successful control efforts involved switching to different classes of broad spectrum drugs for treatment of serious infections. The two classes most commonly chosen are ampicillin-sulbactam and Piperacillin-tazobactam. Jan Patterson noted in their study that decrease in Piperacillin-tazobactam resistance in *Klebsiella* occurred despite a simultaneous increase in Piperacillin-tazobactam use [14]. He drives home the point about decreased ceftazidime use being associated with decreased institutional prevalence of ESBL producing *Klebsiella*.

In a telling article, Rahal and Urban demonstrated that extensive cephalosporin class restriction significantly decreased nosocomial, plasmid mediated cephalosporin pathogens [15]. Al Akayleh goes so far as to state that *Klebsiella* is the most resistant of the entire Gram negative and Gram positive organisms studied [16]. Bhat & Islam et al both suggested that regular microbiological surveillance and in vitro testing would play an important role in guiding the proper empirical antimicrobial therapy in burn patients, preventing multidrug resistance by virtue of using antimicrobials that target specific organisms and decreasing infection-related complications [17, 18]. In Tahlan and Keswani's series, Gentamycin was the most effective drug against *Klebsiella* [19].

Levofloxacin, Tigecyclin and Cefepime + Tazobactam are tested since last 3 years only, and their sensitivity is more than 84%. However their irrelevant use may lead to decreased in sensitivity in future as seen with other antibiotics.

Conclusion

It may be concluded that *Klebsiella* shows a significant change in sensitivity pattern over last 21 years. Optimism for identifying microbiological agents that would solve the problem of

resistance has been replaced with a much more guarded and realistic view of the battle between humans and pathogenic microorganisms. Efforts are now being channelized towards limiting, rather than eliminating resistance by infection control or a proper antibiotic policy which exercises control of irrelevant and excessive use of antibiotics or a combination of the two.

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Evolving resistance of klebsiella species

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