Research Article

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Emerging resistance of higher antimicrobials and growing sensitivity of old antimicrobials against existing infections in burns

Ajay Lunawat*, Rajesh Sharma, Venkatesh Kolla, Shailendra Patel

Department of of general surgery, SAIMS & PGI, Indore, Madhya Pradesh, India

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*Correspondence: Dr. Ajay Lunawat,

E-mail: drajay_lunawat@rediffmail.com

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ABSTRACT

Background: The widespread resistance of microorganisms to antibiotics threatens to be a future medical disaster. Antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections Objective: To analyze the emergence of resistance to higher group of antibiotics and shift of sensitivity towards older group of antibiotics.

Methods: The microbial colonization of wounds was studied from day of admission to date of discharge or date of expiry (2011-2014). The sampling included swabs taken from clinically deep areas of burn wounds prior to cleaning. Culture and sensitivity reports of urine, central line, bronchoscopic suction, catheter tips were not included in samples. Tissue biopsy during excision was included in the samples. Analysis done with the help of Chi-square test.

Results: Initially after burn injury there is predominance of staphylococcal group followed by gram negative organism, after which there is a predominance of gram negative organism. In this study we observed an increase in resistance to higher group of antibiotics like imipenem (p>0.005) and increased susceptibility to polymyxin group of drugs (p<0.005).

Conclusions: It suggests that prevention is better than cure. Developing resistance can be prevented by misuse of antibiotics, premature change of antibiotics and prevention of cross contamination. For effective prevention of developing resistance to higher antibiotics the burn centres should look in and bring a strict antibiotic policy.

Keywords: Emerging resistance, Pseudomonas, MRSA, Polymyxin group

INTRODUCTION

The widespread resistance of microorganisms to antibiotics threatens to be a future medical disaster.^{1,2} Antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections.³⁻⁶ MRSA, methicillin-resistant coagulasenegative Staphylococci, vancomycin-resistant Enterococci, and multiple resistant Gram-negative

bacteria that possess several types of beta-lactamases, including extended spectrum beta-lactamases (ESBL), ampC-betalactamases, and metallobeta-lactamases (MBL), have been emerging as serious pathogens in hospitalized patients. The Gram positive organisms are initially prevalent during hospital stay; then gradually become superseded by gram negative opportunists that appear to have a greater propensity to invade. Pseudomonas aeruginosa is one such difficult-to-treat organism, and reports from the National Nosocomial Infections Surveillance (NNIS) in 1998 indicated that it

then ranked second among the most commonly isolated Gram-negative pathogens. 9-11 Multi-resistance in other Gram-negative bacteria, including strains resistant to carbapenems, is also emerging as a global health issue. 12,13 Outbreaks of P. aeruginosa resistant to most available beta-lactams, aminoglycosides and fluoroquinolones have been reported among cysticfibrosis patients, as well as in burns units and cancer centres. 14-17

Now clinical isolates with mutational fluoroquinolone resistance and metallo-lactamases are being seen with increasing frequency worldwide. Colistin, also known as polymyxin E, is an old antibiotic with significant in vitro activity against some multiresistant Gram-negative pathogens, including P. aeruginosa, A. baumannii and Klebsiella pneumoniae. When the use of a beta-lactam, aminoglycoside, or quinolone is ineffective, the polymyxins, particularly colistin, remain drugs of last resort. 19

Objective: To analyze the emergence of resistance to higher group of antibiotics and shift of sensitivity towards older group of antibiotics.

Inclusion criteria

Patient admitted within 24 hours of burn injury.

Exclusion criteria

- a) Patient admitted after 24 hours or referred from other hospital.
- b) Patient under the age of 18 years and greater than 70% of total burn surface area.

METHODS

The microbial colonization of wounds was studied from day of admission to date of discharge or death (2011-2014). The sampling included swabs taken from clinically deep areas of burn wounds prior to cleaning. Later, swabs were taken on debridement or excision and grafting. The swabs from burn wounds were taken on day zero, 3, 5, 7 and then twice a week till patients are discharged or succumb to burn injuries. Culture and sensitivity reports of urine, central line, bronchoscopic suction, catheter tips were not included in samples. Tissue biopsy during excision was included in the samples. Various culture media are used, e.g. MacConkey agar, blood agar, etc. After incubation for 18-48 h, sensitivity tests performed. Analysis done with the help of Chi-square test.

RESULTS

A total number of 1665 samples of swab were taken of which 615 samples are taken during first five days, and out of 615 samples 305 (49.6%) turned out to be sterile.

over remaining 315 samples Staphylococcus aureus was the commonest micro-organism (13.5%), of which MRSA strains of Staphylococcus constitutes (6.5%), Pseudomonas (9.8%), Klebsiella (7.9%), E. coli (6.5%) were other strains grown and separated during first week.

Table 1: Microorganism organism isolated during first 5 days.

Microbe	Samples	%
Staphylococcus aureus	83	13.5%
Pseudomonas aeruginosa	60	9.8%
Klebsiella species	49	7.9%
E. coli	40	6.5%
Staphylococcus aureus (MRSA)	40	6.5%
Acinetobacter baumanni	23	3.7%
Proteus sp.	15	2.5%
No growth	305	49.6%

Out of remaining 1050 samples which were taken from the burn wounds, after 5 days bacterial isolates were found in 1042 (99.2%) samples and only 8 wound swabs were sterile (0.8%), pseudomonas was leading (38.9%) followed by Klebsiella (26.9%), MRSA (11.6%) Staphylococcus aureus (6.8%), E. coli (3.7%) Proteus sp. (5.9%), Acinetobacter (2.8%).

Table 2: Microbial colonization after 5th day.

Microbe	Samples	%
Pseudomonas aeruginosa	408	38.9%
Klebsiella pneumonia	282	26.9%
Staphylococcus aureus (MRSA)	122	11.6%
Staphylococcus aureus	71	6.8%
E. coli	39	3.7%
Enterobacter sp.	22	2.1%
Proteus sp.	62	5.9%
Acinetobacter baumanni	30	2.8%
Streptococcus sp.	6	0.5%
No growth	8	0.8%

Pattern of antibiotic sensitivity

Among gram positive organism Staphylococcus aureus is 84% sensitive to chloramphenicol, 88.56% sensitive to rifampicin, 99.5% sensitive to vancomycin and 98.7% sensitive to linezolid and marked resistance is observed for penicillin groups and increased resistance pattern is observed for clindamycin (Table 4).

Among gram negative organism, pseudomonas is most common followed by Klebsiella, E. coli, Proteus and Acinetobacter baumanni.

Pseudomonas was markedly resistant to penicillins (96%) piperacillin/tazobactum (77.3%) whereas resistance was 52.4% for amikacin and 63.2% for gentamicin, ceftazidime (92.6%) and tobramycin (71.8%) (Table 3).

In this study, we observed an increase in resistance to the carbepenem group over a period from 2011 to 2014, in 2011 Pseudomonas is 85% sensitive to the imipinem which gradually reduced to 60% by the end of 2014, and in other Gram negative group there is an average of 10% reduction in sensitivity to carbepenem group. During this period we observed an increased sensitivity to the polymyxin group of drugs (90%) (Table 5 & 6).

Klebsiella pneumonia is 92% resistant to penicillin and cephalosporin group of drugs, 75% resistance to co trimoxazole group and shows 73% sensitivity to imipinem and 78% sensitivity to polymyxin group of drugs and 75% sensitivity to tetracycline drug. As

observed over this study period there is increased resistance to imipinem and no change in sensitivity towards polymyxin group.

E. coli is shows similar resistant pattern to penicillin and cephalosporin group but there is also marked resistance observed towards aminoglycosides group of drugs (avg-75%) and 77% sensitive to imipenem and 70% to polymyxin group of drugs. As observed over this study period there is increased resistance to imipenem and no change in sensitivity to polymyxin group.

Proteus and Acinetobacter shows similar sensitivity pattern to imipenem and colistin.

Table 3: Antimicrobial sensitivity pattern of gram negative organisms.

Antimicrobial agent	Sensitivity	Pseudo aerugii N-468		E.coli N-79		Klebsic N-331	ella	Prote N-77	us	Entero N-22	obacter	Acine N-53	tobacter
		N	%	N	%	N	%	N	%	N	%	N	%
AMP	S	17	3.6	12	15.1	25	7.5	0	0	1	5	0	0
Alvii	R	451	96.4	67	84.9	306	92.5	77	100	21	95	53	100
AN	S	224	47.9	18	23	252	76.1	25	32.5	8	36.6	5	9.5
AIN	R	244	52.1	53	67	79	23.9	52	67.5	14	63.4	48	90.5
GN	S	172	36.7	10	12.7	251	75.8	13	16.9	4	18	5	9.5
GIN	R	296	63.3	69	87.3	80	24.2	64	83.1	18	82	48	90.5
CRO	S	0	0	0	0	14	4.2	7	9	0	0	0	0
CKO	R	468	100	79	100	317	95.8	5	91	22	100	53	100
CAZ	S	23	5	2	2.4	15	4.5	5	6.4	1	5	0	0
CAZ	R	445	95	77	97.6	316	93.5	72	93.6	21	95	53	100
CVT	S	32	6.8	12	15.4	79	23.8	0	0	0	0	0	0
SXT	R	436	93.2	57	84.6	252	76.2	77	100	22	100	53	100
TDM	S	128	27.4	19	24	70	21.2	14	18.1	2	6	0	0
TBN	R	340	72.6	50	76	261	79.8	62	81.9	20	94	53	100
DID /E/Z	S	107	22.9	0	0	49	14.8	1	1.3	2	6	1	1.9
PIP/TZ	R	361	77.1	79	100	282	95.2	76	98.7	20	94	52	98.1
CID	S	230	48.6	22	27.8	107	32.3	34	44.1	4	18	0	0
CIP	R	241	51.4	49	72.2	224	67.7	42	56.9	18	82	55	100
IDM (S	349	74.6	61	77	242	73.1	60	77.9	16	72.8	29	52.7
IPM	R	119	25.4	18	23	89	26.9	17	22.1	6	28.2	26	47.3
C	S	113	24.2	28	35.4	110	33.2	23	29.8	10	45.5	16	31.2
С	R	355	75.8	51	64.6	221	66.8	54	70.1	12	54.5	37	69.8
D) (D	S	369	78.4	0	0	259	78.2	0	0	0	0	1	1.9
PMB	R	92	21.2	79	100	72	21.8	77	100	22	100	52	98.1
		n-268	%	n-50	%	n-220	%	n-54	%	n-12	%	n-50	
COL	S	218	81.4	35	70	174	79	29	53.7	8	67.7	47	94
COL	R	50	18.6	15	30	46	21	25	47.3	4	22.3	3	6

AMP-Amoxy, AN-Amikacin, GN-Gentamicin, CRO-Ceftriaxone, CAZ-Ceftazidime, TBN-Tobramycin, PIP/TZ-Piperacillintazobactum, CIP-Ciprofloxacin, IPM-Imipinem, C-Chloramphenicol, PMB-Polymyxinb, COL-Colistin, SXT-Co-Trimoxazole

Table 4: Antimicrobial sensitivity of gram positive organism.

Antimicrobial	Sensitivity		ylococcus s (N-235)	CoNS (N-49)	
agent		N	%	N	%
Р	S	15	6.4	6	12
Г	R	220	93.6	43	88
AMXY/SUL	S	106	45.1	12	41
AMA 1/SUL	R	229	54.9	17	59
CRO	S	35	14.9	7	14.2
CKO	R	200	85.1	42	85.8
0	S	35	20	8	16.3
U	R	137	80	41	83.7
L	S	96	40	17	34.6
L	R	149	60	32	65.4
SXT	S	56	23.8	8	16.3
SAI	R	179	76.2	41	83.7
R	S	207	88	46	93.8
K	R	28	12	3	6.2
Т	S	178	75.7	42	85.7
1	R	67	24.3	7	14.3
С	S	195	84.2	43	87.8
	R	40	5.8	7	12.2
CLN	S	167	71	33	67.3
CLIV	R	68	29	16	32.7
LNZ	S	232	98.7	47	96
LINZ	R	3	1.3	2	4
VA	S	234	99.5	47	96
VA	R	1	`0.7	2	4

P-Penicillin, Amxy/Sul-Amoxycillin and Sulbactum, CRO-Ceftriaxone, O-Oxacillin, L-Levofloxacin, R-Rifampin, T-Tetracycline, C-Chloramphenicol, CLN-Clindamycin, Lnz-Linezolid, VA-Vancomycin, SXT-Co-Trimoxazole, CoNS-Coagulase negative staphylococcus

Table 5: Changing pattern of antibiotic sensitivity for gram negative organism - Antibiotic sensitivity pattern during 2011-2012.

Antibiotics sensitive	Pseudomonas aeruginosa N-226	Klebsiella pneumonia N-178	E. coli N-49	Proteus N-40	Acinetobacter N-24
AMP	4.8% (n-11)	11% (n-20)	24.4% (n-11)	7.5% (n-3)	0
AN	47.7% (n-108)	78% (n-139)	32.6% (n-16)	32.5% (n-13)	16.7% (n-4)
GN	45% (n-102)	76.4% (n-136)	2.6% (n-1)	17.5% (n-7)	16.7% (n-4)
CAZ	6% (n-14)	9% (n-16)	0	10% (n-4)	0
TBN	46% (n-104)	19.7% (n-35)	6.1% (n-3)	22.5% (n-9)	0
PIP/TZ	25.2% (n-57)	15.1% (n-27)	14.2% (n-7)	2.5% (n-1)	4.1% (n-1)
SXT	14% (n-32)	21% (n-38)	10.2% (n-5)	0	0
CIP	53.9% (n-122)	34.2% (n-61)	30.6% (n-15)	25% (n-10)	0
IPM	85% (n-192)	78.1% (n-139)	77.6% (n-38)	77.5% (n-31)	50% (n-12)
PMB	76% (n-172)	78.1% (n-139)	2.1% (n-1)	2.5% (n-1)	4.1% (n-1)
T	-	82.5% (n-147)	57.1% (n-28)	-	-

Table 6: Changing pattern of antibiotic sensitivity for gram negative organism - Antibiotic sensitivity pattern during 2013-2014.

Antibiotics sensitive	Pseudomonas aeruginosa N-242	Klebsiella pneumonia N-153	E.coli N-30	Proteus N-37	Acinetobacter N-29
AMP	2.9% (n-7)	5.3% (n-8)	0	0	0
AN	47.5% (n-115)	73.8% (n-113)	33.3% (n-10)	32.4% (n-12)	3.4% (n-1)
GN	28.9% (n-70)	75.8% (n-116)	16.6% (n-10)	16.2% (n-6)	3.4% (n-1)
CAZ	0	0	0	0	0
TBN	12.8% (n-31)	44.5% (n-68)	30% (n-9)	16.2% (n-6)	0
SXT	0	0	20% (n-6)	8.1% (n-3)	0
PIP/TZ	20.7% (n-50)	15.7% (n-24)	13.3% (n-4)	8.1% (n-3)	3.4% (n-1)
CIP	44.6% (n-108)	30.7% (n-47)	30% (n-9)	14% (n-5)	0
IPM	59.1% (n-143)	68% (n-104)	70% (n-21)	75.6% (n-28)	55.7% (n-16)
COL	89.7% (n-217)	79% (n-121)	90% (n-27)	81% (n-30)	96.5% (n-28)
T	-	75% (n-115)	50% (n-15)	-	-

Table 7: Year wise association of antibiotics with bacterial sensitivity.

		Pseudomonas	Klebsiella	E. coli	Proteus	Acinetobacter	P value
AG	2011-12	106	133	8	10	4	0.734
AG	2013-14	92	115	10	9	1	0.734
PIP/TZ	2011-12	57	27	7	1	1	0.076
PIP/IZ	2013-14	50	24	4	3	1	0.976
IPM	2011-12	192	139	38	31	12	0.393
IPWI	2013-14	143	104	21	28	16	0.393
PMB/E	2011-12	172	139	1	1	1	0.000
PIVID/E	2013-14	217	121	27	30	28	0.000

^{*}Chi-square test was applied

As there is already marked resistance for penicillins, cephalosporins, Co-trimoxazole and tetracycline group (other than E. coli and Klebsiella), we include the above antibiotics which showed increasing resistance pattern over a period of 2011-2014.

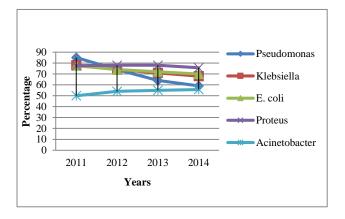


Figure 1: Changing trends of sensitivity of various bacterial isolates to imipinem.

Showing the bacterial sensitivity to imipinem. There is increased resistance observed for imipinem drug over four years for gram negative group of organisms.

From the above comparative Table 7 of year wise association of antibiotics with bacterial sensitivity and analysis done with the help of Chi-square test. It was observed that gram negative organisms are significantly susceptible to (p<0.005) polymyxin group of drugs and there has been a significant increase in resistance to imipinem, piperacillin and aminoglycosides (p>0.005).

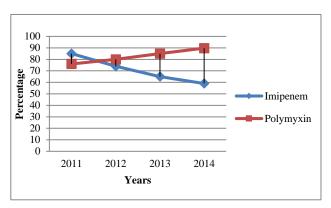


Figure 2: Showing changing trends of pseudomonas sensitivity to antimicrobials.

Shows decreased sensitivity of pseudomonas to impinem and increase sensitivity to polymyxin group of drugs.

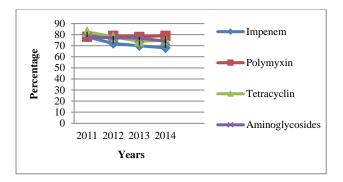


Figure 3: Changing trends of Klebsiella sensitivity to antimicrobials.

Shows changing trends of Klebsiella organism to various antibiotics, increasing resistance to imipinem, tetracycline and aminoglycoside and increasing sensitivity to polymyxin group.

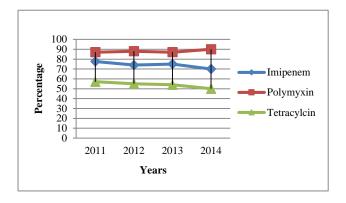


Figure 4: Changing trends of E. coli sensitivity to antimicrobials.

Shows increasing resistance to imipinem and tetracycline and increase in sensitivity to polymyxin group.

DISCUSSION

There is significant change in the antibiograms of the gram negative organism as when compared to the gram positive organisms. Most of the gram positive organisms belong to the MRSA group which are sensitive to the vancomycin, linezolid, rifampicin, chloramphenicol and clindamycin. There is some amount of increase in resistance observed to clindamycin group of drugs over this period. Various studies showed that staphylococcus is resistant to penicillin (97%), clindamycin (77%) tetracycline (57%), rifampicin (54%), chloramphenicol (17%), linezolid (10%) and vancomycin (0%).²⁰ But as compared to our study over this period there is similar resistant patterns with penicillin group but sensitivity to tetracycline (74%), chloramphenicol (84%), rifampicin (88%), LNZ (97%), vancomycin (99%).

Gram negative pathogens continue to cause the most severe infections in burn patients. Morovat et al. reports that P. aeruginosa (44%) followed by MRSA (33%) is the most common organism isolated in burn centers in US.²¹ But according to our study P. aeruginosa followed by

Klebsiella pneumonia followed by Staphylococcus aureus and MRSA is the commonest organisms isolated in our centre.

Various studies shows that pseudomonas is resistant to piperacillin (65.8%), imipinem (62%), ciprofloxacin (60.25%), gentamicin (63%) and ceftazidime (65%).²²⁻²⁷ In our study over all resistance to piperacillin (77.3%), IPM (25%), ciprofloxacin (51%), gentamicin (63.2%) and ceftazidime (95%). But as observed and compared over a period of these four years there is increased resistance to carbepenem group of drugs, in 2011 sensitivity is 85% which reduced to 59.1% in 2014. As time goes on there is increased sensitivity to polymyxin group of drugs (colisitin-89.7%) (p=0.000) and other gram negative organism (85%) (p=0.000) (Figure 1, 2).

Klebsiella, E. coli, Proteus and Acinetobacter do not show significant difference in resistance pattern to carbepenem group and also showed increasing sensitivity to the polymyxin group of drugs.

The causes for increase in drug resistance might be misuse, cross contamination, change in genome of the organisms or premature change of antibiotics. In our centre we send the swabs at regular intervals to prevent misuse of antibiotics and premature change of antibiotics. In a study by Weber et al. showed that the rate of crosscolonization with resistant organisms in 66 critically ill children with severe burns and inhalation injury on ventilator support during a 5 year period was extremely low (3.2 cases per 1000 patient-days) in such a center where housing burn patients in individual nursing units composed of individual isolation rooms, each with its own laminar airflow and should allow all intensive and burn care procedures, including ventilation and operative procedures, to be done within the burn centre itself, or, as a minimum, the facility design should minimize the need to transfer patients out of the burn unit for different aspects of their care.²⁸

CONCLUSION

An effective infection control policy is very much required to reduce or eliminate endemic pathogenic and/or antibiotic resistant organisms, prevent the establishment of antibiotic-resistant organisms as the predominant nosocomial flora of the burn unit, and prevent cross-contamination.²⁹

In developing countries like India cross contamination with resistance microbial flora cannot be prevented because of overcrowding, inadequate sterilization and disinfection practices, gross contamination of the environment, lack of isolation facilities, inadequate hand washing and inadequate resources which are main culprits of cross contamination and increased resistance to higher antibiotics. For effective prevention of developing resistance to higher antibiotics the burn centres should look in and bring a strict antibiotic policy.

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Conflict of interest: None declared

Ethical approval: The study was approved by the

institutional ethics committee

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