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RESEARCH ARTICLE

Emergence of Pan-drug resistance amongst gram negative bacteria! The First case series from India

Abdul Ghafur[1], Vidyalakshmi PR[1], A Murali[2], Priyadarshini K[1], Thirunarayan MA[3]

[1] *Department of Infectious Diseases, Apollo Speciality Hospitals, Chennai, India*
[2] *Department of Infectious Diseases, Apollo Hospitals, Chennai, India*
[3] *Department of Microbiology, Apollo Hospitals, Chennai, India*

ABSTRACT

Objective: Increasing prevalence of carbapenem resistant Gram negative bacteria is a serious clinical and public health challenge. Bacteria resistant to all available antibiotics (Pan Drug Resistance) herald the onset of post antibiotics era. We hereby report clinical profile of 13 patients with pan drug resistant gram negative isolates.

Methods: Retrospective analysis of 13 patients with pan drug resistant gram negative isolates over the last 18 months was done by medical records review. Identification of the isolates and susceptibility testing was done using VITEK auto analyzer in concordance with the corresponding CLSI guidelines.

Results: Out of four patients with bacteremic isolates, three patients received colistin based combination therapy. Though two of these patients had microbiologic clearance, all the three died. Out of the 9 patients with non bacteremic isolates, 4 had infection and 5 had colonization. Three (out of four) were treated with combination therapy including colistin and one patient received colistin monotherapy. All four patients had microbiological clearance. Three patients had clinical cure and were discharged. One patient later developed bacteremia and died.

Conclusion: Infections, particularly blood stream with pan drug resistant organisms has a higher mortality. Urgent studies to reevaluate existing therapeutic options and research into new antibiotic molecules are the need of the hour. *J Microbiol Infect Dis 2014; 4(3): 86-91*

Key words: Pan drug resistance, gram negative bacterial infections, colistin

Tam dirençli gram negative bakterilerin doğuşu: Hindistan'dan ilk vaka serisi

ÖZET

Amaç: Karbapenem dirençli Gram negatif bakterilerin sıklığının artması halk sağlığı ve klinik yaklaşım açısından ciddi bir problemdir. Mevcut bütün antibiyotiklere dirençli (tam dirençli) bakteriler antibiyotik sonrası dönemin başladığının habercisidir. Burada tam dirençli gram negatif suşlarla enfekte 13 hastanın profilini sunmaktayız.

Yöntemler: son 18 ay içerisinde takip edilen tam dirençli gram negatif izolatlarla enfekte olan 13 hastanın kayıtları retrospektif olarak incelendi. Bakterilerin izolasyonu ve duyarlılıkları CLSI rehberine uygun olarak VITEK otomatik analiz cihazı ile yapıldı.

Bulgular: Bakteriyemili hastalardan üçü kolistin içeren kombinasyon tedavisi aldı. Bu hastalardan ikisinde bakteri kandan temizlenmesine rağmen üçü de öldü. Bakteriyemi olmayan dokuz hastadan dördü enfeksiyon beşi ise kolonizasyon olarak kabul edildi. Bu dört hastadan üçü kolistin içeren kombinasyonla ve biri de tek başına kolistin ile tedavi edildi. Bu dört hastada mikrobiyolojik olarak temizlenme sağlandı. Üç hasta klinik olarak iyileşti ve taburcu edildi. Bir hastada daha sonra bekteriyemi gelişti ve öldü.

Sonuç: Özellikle tam dirençli bakterilerle olan kan dolaşımı enfeksiyonları yüksek mortaliteye sahiptir. Şu anda mevcut tedavilerin yeniden gözden geçirecek çalışmalara ve yeni antibiyotik moleküllerini araştırmaya acil olarak ihtiyaç vardır.

Anahtar kelimeler: Tam direnç, gram negatif bakteri enfeksiyonları, kolistin

Correspondence: Abdul Ghafur, Consultant in the department of Infectious diseases, Apollo Speciality Hospital, 320, Padma Complex, Anna Salai, Chennai - 600 035 Email: drghafur@hotmail.com
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INTRODUCTION

The emergence and dissemination of carbapenem resistance among Gram-negative bacteria is a serious public health threat and treatment of patients infected by these bacteria poses therapeutic challenge. Non availability of newer treatment options in fact led to re-evaluation of colistin, a drug introduced into the market half a century ago and then out of use due to its toxicity profile. Now, the long dreaded colistin resistance and hence pan drug resistance scenario among gram negative organism has emerged, leaving very little options to treat patients infected by these bacteria.

There are plenty of laboratory studies on the prevalence of colistin resistance. As per a review published in 2007, percentage of polymixin resistance in 11 invitro studies ranged from 1.4%-5% and pan drug resistance rate of 1.9%-3.3%.1 A 2001 Spanish study reported a very high colistin rate of 13.1% in *A. baumannii*.[2] A study from Kuwait reported 12% resistance among *Acinetobacter* isolates.3 The SENTRY study by Gales et al, published in 2006, reported 1.3% polymixin resistance and 0.08% pan drug resistance amongst *Pseudomonas* and 2.1 and 0.3% respectively in *Acinetobacter* spp. Out of the 8188 *Klebsiella* isolates tested 1.8% were resistant to colistin.[4]

A study from North India (Taneja et al) analyzed 224 isolates of *A. baumannii*. Of the total isolates, 3.5% and 16% of the carbapenem resistant MDR strains were found to be pan-drug resistant (PDR) i.e., resistant to both tigecycline and colistin.[5] Chand Wattal et al from a tertiary care hospital in North India showed that 8% of the *pseudomonas* was colistin resistant.[6] There are no studies from India outlining both the clinical and microbiological characteristics of pan drug resistant isolates. This data if available will be extremely useful from a clinical and public health view point. We hereby report clinical profile of 13 patients with pan drug resistant gram negative bacterial isolates from various sites.

Objective of the study

To elaborate on the clinical characteristics, management and outcome of patients with culture positivity for PDR gram negatives isolates.

METHODS

Retrospective analysis of 13 patients with pan drug resistant gram negative isolates, identified over a period of one and a half years was done by medical records review, in a tertiary care oncology and stem cell transplant centre in India.

The isolates were tracked from the microbiology laboratory. Susceptibility testing was performed using VITEK2 compact. The isolates were tested against piperacillin-tazobactam, gentamicin, amikacin, netilmycin, ceftazidime, cefoperazone-sulbactam, cefepime, cefepime/tazobactam, imipenem, meropenem, ciprofloxacin, trimethoprim/sulfamethoxazole and tigecycline. While clear cut CLSI guidelines on breakpoints are available for Enterobacteriaceae and *Pseudomonas* for to most antibiotics, there is no defined breakpoint for cefoperazone-sulbactam and cefepime/tazobactam. Breakpoints of cefoperazone and cefepime were applied for them respectively. Antibiotic discs for these drugs were obtained from Hi Media Lab India. Colistin susceptibility was done using VITEK 2 compact for all isolates and for some isolates MIC was assessed by E test according to availability. *Pseudomonas* isolates were considered to be resistant to colistin if the MIC is > 8 and *Acinetobacter* if MIC > 4.[7] There is no available colistin breakpoint for Enterobacteriaceae as per CLSI guideline, so EUCAST breakpoints were followed (S ≤2; R>2).[8]

Acinetobacter baumannii, *Pseudomonas* and Enterobacteriaceae were considered to be Pan drug resistant (PDR) if isolates were resistant to all classes of anti pseudomonal agents. In addition non-pseudomonal isolates were tested against tigecycline.[1] Hospital identification numbers of patients who had a positive culture with Pan drug resistant gram negative bacteria, between January 2012 and May 2013, were collected from Microbiology laboratory and their medical records were tracked and analyzed. Data for variables like age, sex, co morbidities, ICU stay, presence of indwelling devices and prior antibiotic exposure were looked into. APACHE score, Charlson's co morbidity index and Pitt's bacteremic score was also calculated. Outcome, including 28 day mortality was analyzed.

RESULTS

A total of thirteen patients with pan drug resistant (PDR or possible PDR) isolates were analyzed. Bacteremic and non bacteremic isolates were analyzed separately. Four patients had bacteremia with possible pan drug resistant gram negative organisms. Nine possible PDR gram negative bacteria were isolated from sites other than blood. Four of them had infection due to the organism (two UTI, one SSTI and one pneumonia) and five were colo-

nized. The demographic, clinical and microbiological profile is detailed in Table 1. Three out of four patients had carbapenem exposure and three had colistin exposure during the same admission. Three patients had an indwelling device at the time of bacteremia. Three patients were treated and one was not willing to continue treatment due to personal reasons. All the patients who were treated received combination therapy with colistin, carbapenem, tigecycline. Two patients had microbiological clearance while cultures were not repeated in the other two. Three of them expired and one could not be followed up.

Table 1.1. Clinical profile of patients with PDR bacteremia

Avg Age	S9.S	Prior hospitalisation	3 out of 4
Avg APACHE II score	14.75	Length of stay in ICU prior to colistin resistance	25.75
Avg Charlson's comorbidity index (age adjusted)	4	Indwelling devices present in	3 out 4
Avg Pitt's bacteremic index	3.75	Tool leucocyte count	1 neutropenic (200) & 3 non neutropenics (avg- 8433)
Diagnosis	3 Malignancy (1 hematological, 1 meningioma & 1 ca larynx) and 1 trauma	Treatment	3 treated (combination therapy) & 1 not treated (discharge against medical advice)
Prior positive culture in the same hospitalisation	3 out 4 had a carbapenemase producer	Antibiotics (No.Of days)	Patient 1. Colitin (5), meropenem (5), tigecycline (5) Teicoplanin (5) Patient 2. Colistin (7), tigecycline (5) Patient 3. Colistin (12), meropenem (9) tigecycline (12)
Exposure to carbapenem & colistin in the same admission	Carbapenem- 3 out of 4 Colistin 2 out of 4	Microbiological clearance	2 cleared & 2 cultures not repeated
Source	1 lung, 1 gut translocation & unclear	Outcome	3 expired and 1 not followed

Table 1.2. Clinical profile of patients with PDR (non-bacteremic)

Avg Age	55	Prior hospitalisation	8 out of 9
Avg APACHE II score of infected patients	18	Length of stay in ICU prior to colistin resistance	20.44
Avg Charlson's comorbidity index (age adjusted) of infected cases	6	Indwelling devices present in	6 out of 9
Diagnosis	3 malignancy, Strauna, 1 myesthenia gravis	Toed leucocyte count (infected patients)	11,112
Prior positive culture in the same hospitalization	5 out of 9 had a carbapenemase producer	Treatment	3 Combination therapy & 1 monotherapy
Exposure to carbapenem & colistin in the same admission	Carbapenem-5 Colistin-7	Antibiotics (No. of days)	Patient 1. Colistin (5), meropenem (5) Patient 2. Colistin (12) Patient 3. Colistin (9), cefepime (5), teicoplanin (13) Patient 4, Colistin (14), doripenem (12), tigecycline (14), rifampicin (6)
Infection/Colonisation	4 infection & 5 colonisers	Microbiological clearance	5 out of 9 repeated and cleared
Site (infection)	2 urine, 1 wound, 1 lung	Outcome of infected patients	3 stable & discharged and 1 died

Table 1. The demographic, clinical and microbiological profile of patients.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13
Age	57	70	57	37	27	48	45	44	34	26	67	66	77
Sex	F	M	M	F	M	M	M	F	M	F	M	M	M
Diagnosis	Ca larynx	Ca bladder & ureter	Myasthenia gravis	AML	Traumatic brain injury	Traumatic brain injury	Traumatic brain injury	Meningioma	Traumatic brain injury	Traumatic brain injury	Meningioma	NHL	Traumatic brain injury
Site of isolate	Blood	Urine	Urine	Blood	Tracheal secretion	Sputum	Urine	Tracheostomy tube	Tracheostomy tube	Wound	Blood	Wound	Blood
Oraganism isolated	*Klebsiella*	*Enterobacter*	*Klebsiella*	*Klebsiella*	*A.baumannii*	*P.aeruginosa*	*Klebsiella*	*P.aeruginosa*	*P.aeruginosa*	*Klebsiella*	*A.baumannii*	*Klebsiella*	*Klebsiella*
CCU admission	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
APACHE II SCORE	20	N.A	13	N.A	16	N.A	10	15	16	24	16	28	9
Charlson's comorbidity index	4	4	2	2	2	8	6	2	3	12	5	15	5
Pitt's bacteremic score	4	N.A	N.A	0	N.A	N.A	N.A	N.A	N.A	N.A	5	N.A	6
Previous hospitalisation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No data	Yes	Yes	Yes	No
Previous positive cultures	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes
Previous carbapenem/ colistin therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Length of stay in CCU	2	0	45	0	16	0	26	42	34	18	56	3	45
Infection/Coloniser	Infection	Infection	Infection	Infection	Coloniser	Coloniser	Coloniser	Coloniser	Infection	Coloniser	Infection	Infection	Infection
Treatment	Not treated	Colistin+ meropenem	colistin	Colistin+ meropenem+ tigecycline+ teicoplanin+ rifampicin	Colistin+ aztreonem+ teicoplanin	Not treated	Not treated	Treated for meningitis	Colistin+ teicoplanin+ cefepime	Ceftriaxone	Colistin+ tigecycline	Colistin+ doripenem+ tigecycline+ rifampicin	Colistin+ meropenem+ tigecycline
Micro clearance	Not repeated	cleared	Cleared	Not repeated	cleared	Not repeated	Not repeated	Not repeated	Cleared	Not repeated	Other bacteria	Not repeated	Not repeated
Overall outcome	AMA	Discharged	Discharged	Expired	AMA	Discharged	Discharged	Discharged	Discharged	Discharged	Expired due to subsequent bacteremia	Expired	Expired

Out of the nine patients with non bacteremic possible PDR isolates, four had infection and five were colonized (two patients had UTI, one had skin and soft tissue infection and one had pneumonia). Eight out of the nine patients with possible PDR colonization or infection had prior hospitalization. Five patients had prior carbapenem exposure and seven had colistin exposure. Three (out of four) were treated with combination therapy with colistin and one or more of the following agents (carbapenem, tigecycline, rifampicin, cefepime). One patient received monotherapy with colistin. All four patients had microbiological clearance. Three patients had clinical cure were discharged. One patient progressed to bacteremia and died (Table 2).

Microbiological data of PDR isolates

Three out of four isolates from blood were *Klebsiella pneumoniae* and the other was *Acinetobacter baumannii*. Colistin MIC by E test was available for three of them. Out of the three urinary isolates two were *Klebsiella pneumoniae* and one was Enterobacter. Both the isolates from the wound were *Klebsiella pneumoniae*. *Pseudomonas* was the commonest amongst the respiratory isolates (three out of four). The other was *Acinetobacter baumannii*. Colistin MIC by E test was available for four out of the nine isolates.

DISCUSSION

Treatment of extremely drug resistant bacteria poses a serious challenge to patients and the treating physicians. Antibiotic development pipe line against Gram negative bacteria is nearly dry and no new antibiotic is expected at least for the next few years.[9] Colistin is the drug of choice for treatment of patients having severe infections due to these bacteria. Colistin usage, in parts of the world with high prevalence of XDR Gram negative bacteria has resulted in reports of colistin resistant.[10-12] As expected with any other antibiotic, increased usage of colistin results in increasing reports of colistin resistance.[13,14] Only a few clinical studies have been published outlining clinical characteristics and the outcome of patients with PDR isolates, mostly from Greece.[1,10,15-18]

It should be noted that some of the earlier reports on PDR bacteria haven't included colistin susceptibility and may not be pan drug resistant as per the new international expert proposal.[19-21] According to this new nomenclature, all antibiotics of the suggested groups should be tested before reporting as pan drug resistant, making many earlier reports of "PDR" to "possible PDR". We did not test for fosfomycin and chloramphenicol sensitivity of our isolates and so all our PDR isolates, by the new definition becomes possible PDR. Beno et al reported 9 cases and Falagas et al published two series (7 and 28 patients), Tsioutis et al. reported 21 patients with pan drug resistant gram negative bacteria.[10,15-18]

In our study 4 patients had bacteremia, 2 had UTI, one had pneumonia and one had SSTI. Three out of four patients with bacteremia and 8 out of 9 patients with non-bacteremic isolates had prior hospitalization, an important risk factor, pointed out in other series as well.

There is limited evidence from two previous clinical studies that isolation of a PDR organism is often preceded by isolation of an organism susceptible only to colistin.[15,16] In our study, three out of four patients with possible PDR bacteremia had prior infections with colistin sensitive, carbapenem resistant organism for which they received colistin, tigecycline and carbapenem. In patients with non bacteremia infections, three out of four patients had a positive culture with a carbapenem resistant organism. All the four had prior colistin exposure. Two out of four had carbapenem exposure. One patient was exposed to ciprofloxacin.

Three out of four patients with possible PDR bacteremia were treated and one didn't continue treatment due to personal reasons. All the three received combination therapy with a colistin based regimen. One patient received colistin and meropenem, second patient received colistin, meropenem and tigecycline and the third patient received colistin, meropenem, tigecycline, teicoplanin and rifampicin. Three out of four patients with non bacteremic infections received combination therapy. One patient received colistin and meropenem, one received colistin, cefepime-tazobactam and teicoplanin and the third one received colistin, doripenem, rifampicin and tigecycline. One patient received colistin monotherapy. Beno et al study did not provide details on antibiotic therapy used to treat PDR infections in their series.[15] In a study by Falagas et al on PDR infections with *Klebsiella* and *Pseudomonas* patients were treated with colistin combined with carbapenem, BLBLI, quinolone, aminoglycoside and co-trimoxazole in varying combinations.[16] In another study by Falagas et al, the possible PDR patients were treated with different combination of antibiotics.[10] In a study from Greece, nine (42.9%) patients received monotherapy for the treatment of the PDR infection; six patients received tigecycline, two patients received meropenem, and one patient

received colistin. Twelve (57.1%) patients were treated with a combination of antibiotics, which was mainly based on colistin plus meropenem or doxycycline or rifampicin or ciprofloxacin.[18]

Cure rate in Beno et al study was 5 out of the 9 and in Falagas et al study was 5 out of 7. In the Falagas et al study overall in-hospital mortality was 41.7% and infection related mortality 33.3%.[10,15] In the study by Tsioutis et al. 16 patients were cured and 5 died. Three out of ten patients treated with a colistin containing regimen died. All seven patients treated with tigecycline were cured and they were also found to have a shorter duration of stay after infection onset.[18] In our 4 patients with bacteremia 3 died, though repeat cultures were negative in two. One patient with bacteremia could not be followed up and so outcome is not known. However amongst the non bacteremic isolates 3 out of 4 were discharged and 1 expired. Reported mortality in other studies was 50%-100% for patients with PDR *Klebsiella* and 20% for *Pseudomonas*. In our study all the 3 patients with PDR isolates in the blood were *Klebsiella pneumoniae* and carried a very high mortality.[15,16]

CONCLUSION

Infection due to Pan drug resistant bacteria is a therapeutic challenge as this heralds the dawn of post antibiotic era with clinicians left with no antibiotic option. Our study is the first published one on the topic from India, providing clinical characteristics of patients with possible PDR isolates. The data also highlights the need for laboratory and clinical studies on combination therapy in the treatment of PDR and XDR Gram negative bacterial infections. Prospective studies on the topic are required on an urgent basis. Findings of such trials can guide clinicians to choose the best therapeutic option. With high carbapenem resistance rates and increasing colistin usage in many tertiary care centers in India, clinicians and laboratories should look out for colistin resistance through appropriate laboratory tests. Early detection of colistin resistance helps in initiating proper infection control measures to prevent the spread of these bacteria in health care institutions.

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