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RESEARCH ARTICLE OPEN ACCESS

Antibiogram of Gram Negative Bacteria Isolated from the Skin and Soft Tissue Infections a Guide for Empirical Therapy to the Clinicians

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Abstract

The antibiogram gives the periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the hospitals microbiology laboratory. Antibiogram can be of great use in assessing the local susceptibility rates and can serve as a tool in designing the empirical antibiotic therapy and also in monitoring the resistance trends over time within in an institution. Pus samples from various clinical conditions like abscess, cellulitis, necrotizing fasciitis, wound infections; diabetic foot ulcers were included in the study. A total of 1124 positive cultures were obtained out of which 736 yielded various Gram negative organisms and 488 were Gram positive organisms. Only Gram negative organisms were considered in the study as gram negative organisms are common etiological agents of skin and soft tissue infections and pose a great challenge to the treating physician as they are known to develop a high antimicrobial resistance. The organisms isolated in our hospital were *Pseudomonas aeruginosa* (192), *Klebsiella pneumonia* (173), *Escherichia coli* (168), *Citrobacter* species (117), *Acinetobacter* species (47), and *Proteus* species (39). In our study which aims at formulating an empirical therapy for Gram negative organisms the drugs with highest sensitivity were Imipenem (51%), Amikacin (43%), Meropenem (38%), Tobramycin (36%), and Ciprofloxacin (34%) Gentamicin (34%), Netimicin (33%), Cotrimoxazole (32%), Piperacillin (28%),Tetracycline (28%), Ceftazidime (28%), Levofloxacin (26%), Ceftriaxone (26%), Colistin (22%), Carbenecillin (21%), Cefoperazone (21%), Cefoperazone +Sulbactum (21%), Azonetrem (21%), Cefipime (20%), Cefuroxime (17%), Cephaxlein (15%), Ampicillin (12%), Amoxyclav (10%). With the knowledge of most commonly isolated organisms causing SSTIs and their antimicrobial susceptibility patterns the clinicians can start the most likely antibiotic and can change accordingly once the sensitivity report is available.

Keywords: Resistance, Antibiogram, Empirical therapy, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*

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INTRODUCTION

Antimicrobial resistance is a global challenge. Survival of the fittest strategy by acquisition of resistant genes like any other living being has been used by bacteria. This can be slowed down by reducing the selective pressure on bacteria causing infectious diseases[1].

The antibiogram gives the periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the hospitals microbiology laboratory. Antibiogram can be of great use in assessing the local susceptibility rates and can serve as a tool in designing the empirical antibiotic therapy and also in monitoring the resistance trends over time within in an institution[2].

Antimicrobial resistance (AMR) in Gram negative bacteria (GNB) has been a significant cause of severe infections across the world, with increasing morbidity and mortality rates, prolonged hospitalization, significant increase in the cost of medical care and also increased rates of hospital acquired infections[3].

Skin and Soft tissue infections involve the microbial invasion of the skin and underlying tissues and range from mild infections such as pyoderma to serious life threatening infections such as necrotizing fasciitis[4].

The risk factors may be patient related to age more than 60 years, malnutrition, diabetes, immunosuppression, skin colonization with Methicillin Resistant *Staphylococcus aureus* (MRSA), presence of skin diseases, smoking and obesity. Procedure related risk factors like improper surgical scrub, inadequate skin antisepsis, prolonged operative time, inadequate antimicrobial prophylaxis, poor perioperative glycemic control surgical drains, inadequate disinfection and sterilization, emergency procedure and perioperative shaving [5].

There is a significant variation in the resistance profile of the pathogenic bacteria across the world though AMR is rising globally. It is therefore necessary to monitor the rates of AMR in the clinically important pathogens across the world. The changing trends in resistance pattern over time have to be tracked regularly in order to guide the appropriate therapeutic strategies to combat infections due to drug-resistant pathogens. Many large-scale surveillance studies are being conducted to monitor AMR across the globe. Studies have reported that the burden of AMR is high in Asian countries. However, surveillance studies in India being negligible, incidence rates and real burden of AMR in India is underreported. The study for monitoring AMR trend is a global surveillance program intended to monitor the efficacy of antimicrobials against GNB.[6].

Hence the purpose of this study is to determine the current antimicrobial sensitivity pattern of Gram negative bacteria causing skin and soft tissue infection and formulate a comprehensive empirical antibiotic approach for managing the patients with skin and soft tissue infections caused by Gram negative organisms.

MATERIAL AND METHODS

The study was carried out in the department of Microbiology (BLDE (DU) Shri B M Patil Medical College Hospital & Research Centre Vijaypur Karnataka India) from January 2019 to December 2019. All the cultures yielding Gram negative organisms were included in the study. Patients of both the sexes irrespective of age group admitted in the Department of General Surgery, Orthopedics, and Obstetrics for skin and soft tissue infections were included in the study.

Pus samples from various clinical conditions like abscess, cellulites, necrotizing fasciitis, wound infections; diabetic foot ulcers were included in the study. A total of 1124 positive cultures were obtained out of which 736 yielded various Gram negative organisms and 488 were Grampositive organisms.

Sample collection

The lesions were cleaned with sterile normal saline. Special care was taken to avoid contamination by normal flora of skin or mucus surface, where possible pus was aspirated or exudates collected. The specimens were transported in sterile, leak-proof containers. Pus samples were also collected aseptically using sterile swabs.

Sample processing

Swabs were inoculated on to Blood agar and Mac-Conkey's agar plate. The plates were incubated aerobically at 37°C overnight. The isolates were identified by Gram staining, Colony morphology, Standard biochemical tests

like Oxidase, Catalase, Indole, Methyl red, Vogues Proskauer test and Hydrogen sulphide production test.

The antimicrobial susceptibility testing was performed by modifiedKirby-Bauer disc diffusion technique following clinical and laboratory standards institute guidelines[7].

The antibiotics tested were Ampicillin Amoxyclav, Cefuroxime, Tetracycline, Gentamicin, Cotrimoxazole, Ciprofloxacin, Cephalexin, Amikacin, Lomefloxacin, Netimicin, Tobramicin, Piperacillin, Ceftriaxone, Ceftazidime, Imipenem, Meropenem, Levofloxacin, Cefepime, Azonetrem, Cefoperazone +Sulbactum, Cefoperazone, Carbenecillin, Colistin. Antibiotics from all classes were selected.

RESULTS

A total of 1124 cultures yielded growth, out of which 736 were various Gram negative organisms and 488 were Gram positive organisms. Gram negative organisms were considered for the study. All the isolates were identified by the Gram Stain and biochemical reactions.

Out of 736 organisms isolated, 192 were *Pseudomonas aeruginosa*, 173 were *Klebsiella pneumonia*, 168 were *Escherichia coli*, 117 were *Citrobacter* species, 47 were *Acinetobacter* species, *and 39 were Proteus* species(Table No 1).

Table 1. Bacteriological profile of Pus Samples

No.	Gram negative isolates	No of Isolates
1	*Pseudomonas aeruginosa*	192
2	*Klebsiella pneumonia*	173
3	*Escherichia coli*	168
4	*Citrobacter* species	117
5	*Acinetobacter* species	47
6	*Proteus* species	39
	Total	736

Table 2. Sensitivity Pattern of Gram Negative Bacteria

	E. coli Spp. (N= 47)%	*Acinetobacter Spp.* (N= 173)%	*Klebsiella Spp.* (N= 192)%	*Pseudomonas aeruginosa* (N= 117)%	*Citrobacter Spp.* (N= 168)%	*Proteus Spp.* (N= 39)%
Ampicillin	12	19	13	23	3	13
Amoxyclav	11	21	36	11	3	15
Cefuroxime	20	21	29	23	6	15
Tetracycline	30	28	29	23	22	18
Gentamicin	60	30	34	22	22	26
Cotrimoxazole	38	26	8	23	25	13
Ciprofloxacin	33	28	36	46	17	31
Cephalexin	18	23	40	23	3	13
Amikacin	71	49	40	30	26	46
Lomefloxacin	29	55	31	23	26	36
Netilmicin	29	32	28	32	31	28
Tobramicin	59	34	22	39	9	23
Piperacillin	26	32	23	39	12	18
Ceftriazone	27	26	57	26	10	23
Ceftazidime	31	36	35	29	15	21
Imipenem	46	51	36	53	42	62
Meropenem	35	43	34	43	32	51
Levofloxacin	29	60	42	5.2	25	26
Cefipime	38	4	40	4.2	6	26
Azonetrem	33	13	40	0	12	13
Cefoperazone+ Sulbactum	40	9	13	0	11	8
Cefoperazone	39	9	36	0.5	11	10
Carbenecillin	-	-	-	21	-	-
Colistin	-	-	-	22	-	-

The antibiotics tested were Ampicillin Amoxyclav, Cefuroxime, Tetracycline, Gentamicin, Cotrimoxazole, Ciprofloxacin, Cephalexin, Amikacin, Lomefloxacin, Netilmicin, Tobramicin, Piperacillin, Ceftriazone, Ceftazidime, Imipenem, Meropenem, Levofloxacin, Cefipime, Azonetrem, Cefoperazone+Sulbactum, Cefoperazone, Carbenecillin, Colistin. Table 2 shows the sensitivity pattern of all the gram negative organisms.

Table 3 shows the total sensitivity of all the antibiotics used in the treatment of Gram Negative Bacteria in decreasing order. Imipenem (51%), Amikacin (43%), Meropenem(38%), Tobramycin (36%), Ciprofloxacin (34%), Gentamicin (34%), Netilmicin(33%), Lomefloxacin (32%), Cotrimaxazole (28%), Piperacillin (28%), Tetracycline (28%), Ceftazidime (28%),Levofloxacin (26%), Ceftriaxone (26%), Colistin(22%), Carbenecillin (21%), Cefoperazone (21%), Cefoperazone + Sulbactum (21%), Azonetrem (21%),Cefipime (20%), Cefuroxime (17%), Cephalexin(15%), Ampicillin (12%), Amoxyclav (10%).

Table 3. Total Sensitivity of all the Antibiotics used in the treatment of Gram Negative Bacteria in decreasing order

Antibiotic	Sensitivity %
Imipenem	51
Amikacin	43
Meropenem	38
Tobramycin	36
Ciprofloxacin	34
Gentamicin	34
Netilmicin	33
Lomefloxacin	32
Cotrimaxazole	28
Piperacillin	28
Tetracycline	28
Ceftazidime	28
Levofloxacin	26
Ceftriaxone	26
Colistin	22
Carbenecillin	21
Cefoperazone	21
Cefoperazone+ Sulbactum	21
Azonetrem	21
Cefipime	20
Cefuroxime	17
Cephalexin	15
Ampicillin	12
Amoxyclav	10

DISCUSSION

The organisms isolated in our hospital BLDE (DU) Shri B M Patil Medical College Hospital & Research Centre Vijaypur Karnataka India were *Pseudomonas aeruginosa* (192), *Klebsiella pneumonia* (173), *Escherichia coli* (168), *Citrobacter* species (117), *Acinetobacter* species (47), *and Proteus* species (39) (Table No 1).

Our study which aims at formulating an empirical therapy for Gram negative organisms the drugs with highest sensitivity were Imipenem (51%), Amikacin (43%), Meropenem (38%), Tobramycin (36%), and Ciprofloxacin (34%) Gentamicin (34%), Netimicin (33%), Cotrimoxazole (32%), Piperacillin (28%), Tetracycline (28%), Ceftazidime (28%), Levofloxacin (26%), Ceftriaxone (26%), Colistin (22%), Carbenecillin (21%), Cefoperazone(21%), Cefoperazone + Sulbactum (21%), Azonetrem (21%), Cefipime (20%), Cefuroxime (17%), Cephaxlein(15%), Ampicillin (12%), Amoxyclav (10%) (Table No 3)

Gram negative organisms recovered from Skin & Soft tissue infections among patients were more susceptible to Carbapenems (Imipenem, Meropenem), Aminoglycosides (Amikacin, Tobramycin, Gentamicin) followed by Ciprofloacin and Cotrimoxazole, Piperacillin, 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone, Cefotaxime); Colistin, Carbenecillin

Table 4. Drugs used as Empirical therapy

Amikacin	(Injection)
Gentamicin	(Injection)
Tobramycin	(oral&injection)
Netimicin	(oral&injection)
Ceftazidime	(injection)
Ceftriaxone	(injection)
Cefotaxime	(injection)
Cefipime	(injection)
Cephalexin	(oral)
Cefuroxime	(oral)
Cefoperazone-Sulbactum	(injection)
Piperacillin	
Cotrimoxazole	(oral)
Colistin	(injection)
Imipenem	(injection)
Meropenem	(injection)

4th Generation Cephalosporins (Cefepime), β lactam/ β-lactamase inhibitor combinations (Cefoperazone-Sulbactaum).

After discussing with the clinicians mainly surgeons a list of first line of drugs was drawn Aminoglycosides (Amikacin, Gentamicin, Tobramicin and Netimicin) which are injectables were given the first priority followed by the 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone, Cefotaxime) then by the 4th generation Cephalosporins (Cefepime) which are available orally also then by β-lactam/ β-lactamase inhibitor combinations (Cefoperazone-Sulbactam) then by the Colistin and keeping the Carbapenems (Imipenem and Meropenem) as reserved drugs both in terms of efficiency and cost though they have highest sensitivity. So list of drugs used as Empirical therapy in the treatment of Gram negative infections is as follows:

Hospital antibiogram can be a guiding tool for empirical therapy and can also track the emergence of resistance among the bacterial isolates in the nosocomial environment. There can be a wide variation in the manner in which the antibiograms are formulated and reported which results in intra and inter hospital comparisons[8].

Though the treatment may be dependent on the culture, empirical therapy is necessary and should be designed so that it can cover the both Gram positive and Gram negative organisms till the culture report is available. Surveillance of the antibiotic sensitivity should be done on regular basis as the pathogens and their sensitivity keep on changing and also vary from unit to unit, hospital to hospital[9,10].

It is very much essential that all the clinicians should understand the importance of antibiotic susceptibility testing. Always there has to be mutual sharing of expertise, cooperation and collaboration between the clinicians and microbiologist so that there can be optimum and appropriate use of antibiotics in the management of infectious diseases. Though many studies of regional antibiogram have been reported from India, there is a need for nationwide statics to be generated and made available to the clinicians[11]. An ICMR guideline 2019 for the use of antimicrobials is already available for guiding the empirical therapy but the local antibiogram of the hospital will give exact sensitivity pattern which can have a day to day application.

But again it is the treating physician/ surgeons decision to choose the class of antibiotic as the antibiotic policy has many limitations and also the patient factor needs to be considered including the type and severity of infection, the infecting organism, past antibiotic history.

Bacterial antimicrobial resistance is a great challenge to the medical community and also to the patients in terms of increase in morbidity, mortality and economic burden. Political involvementie the health ministry has to come forward with strict implementation of national antibiotic policies with the cooperation of private corporate setups.

Clinician should not be pressurized to write an antibiotic on patient's demand of early recovery; this will be possible only when the patient is aware of the judicious use of antibiotics which can be achieved through public education or campaigns just like other national health education programs[12].

It is quite essential to define an antimicrobial stewardship program as an ongoing effort by an health care institution to optimize the antimicrobial use among hospitalized patients to improve patient outcomes, ensure cost effective therapy and reduce adverse squeal of antimicrobial use[11].

CONCLUSION

The most commonly isolated gram negative organisms from the skin and soft tissue infections are *Pseudomonas aeruginosa, Klebsiella pneumonia*,*Escherichia coli, Citrobacter species, and Acinetobacter species*. The empirical therapy for the infections caused by these organisms are Aminoglycosides(Amikacin, Gentamicin, Tobramicin and Netimicin), 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone, Cefotaxime),4th generation Cephalosporins (Cefepime), β-lactam/ β–lactamase inhibitor combinations (Cefoperazone-Sulbactam),Colistin and keeping the Carbapenems(Imipenem and Meropenem) as reserved drugs both in terms of efficiency and cost though they have highest sensitivity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution tothe work, and approved it for publication.

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ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY

All data generated or analysed during this study are included in the manuscript.

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