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Isolation and Identification of ESBL producing Enterobacteriaciae from Blood samples received at Dr. Prabhakar Kore Hospital and MRC Belagavi

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A B S T R A C T

Aim : 1. To isolate the Enterobacteriaceae from Blood samples
2. To identify ESBL producers from Blood samples by Phenotypic method

Methods and Materials : The research was carried out within the Department of Microbiology, Jawaharlal Nehru Medical College, KAHER, Belagavi, from January 2024 to December 2024. In the current study, different organisms have been found in Blood samplesreceived in the Department of Microbiology Belagavi between January 2024 and December 2024 at the Department of Microbiology, JNMC, KAHER Belagavi. Kirby bauer disc diffusion is a method for assessing antibiotic susceptibility conforming to guidelines issued by CLSI.

Results : A total of 120 samples were processed at this time, and of those 120 samples, (59%) 70 were found to have NOGC, (08%) 10 were found to have GPC, (13%) 16 were found to have Klebsiellaspp, (03%) 04 found to have Escherichia coli, (02%) 02 were found to have Citrobacterspp, (06%) 07 were found to have Salmonella spp, (09%) 11 were found to have Acinetobacter spp.A Twenty Nine (29) Enterobacteriaciaewere processed in total. at this time, & of those 29 Enterobacteriaciae isolated, in 29 isolates, 25 are ESBL Producers and 04 Non-ESBL producers.

Conclusion:The synthesis of ESBL, beta lactamase enzymes was examinedin 40 different Enterobacteriaciae strains. Production of this enzyme is an important mechanism of resistance among Escherichia coli and Klebsiellapnemoniae, Salmonella spp, Citrobacter Spp.

Keywords : Escherichia coli, Klebsiella pneumoniae.

INTRODUCTION:

Extended-spectrum beta-lactamases, or ESBLs, have been found in most of places worldwide. Recent studies have revealed how common ESBLs and other germs resistant to multiple drugs are in Asia, North America, and Europe. Still, they have been found in other groups of the Enterobacteriaceae family, such as Proteus, Enterobacter, Salmonella, Serratia, and Citrobacter. (1)

Extended-spectrum β-lactamazes (ESBLs), enzymes carried by plasmids, can break down oxyimino β-lactams. A lot of different types of Enterobacteriaceae bacteria have them, like Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae. ESBLs can make bacteria resistant to aminoglycosides, trimethoprim, sulfamethoxazole, and quinolones, among other drug families. They can also make bacteria resistant to penicillins, aztreonam, and cephalosporins. Multidrug resistance among *Klebsiella* spp. and ESBL-producing *E. coli* isolates presents a serious clinical concern, since patients infected with these strains have few treatment options and a high chance of not responding to treatment. (2,3,4)

One of the main threats posed by multidrug-resistant bacterial isolates is extended-spectrum b-lactamases, or ESBLs. After being found in early 1980s, they have been characterized continuously more globally, and they are now well-known among Enterobacteriaceae isolates in almost every European nation, both in the nosocomial and community settings. The present European ESBL epidemiology is the main topic of this review. The composition of mobile genetic components including blaESBL genes and the population structure of isolates that produce ESBL are examined. (7)

Gram-negative bacteria like Klebsiella pneumoniae and Escherichia coli make longer spectrum beta-lactamases (ESBL). Other genera of bacteria like Serratiamarcescens, Shigelladysenteriae, Proteus, Burkholderiacepacia, Salmonella, Pseudomonas aeruginosa and Enterobacter sp. also make ESBL. (8

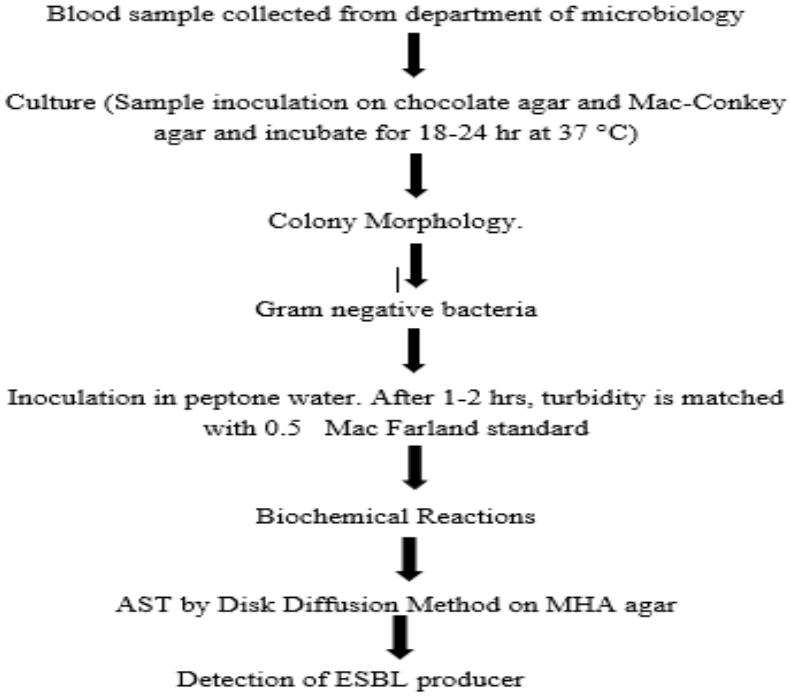
OBJECTIVES:

1. To isolate the Enterobacteriaceae from blood samples.
2. To identify ESBL producers from Enterobacteriaceae isolated from blood samples by phenotypic method.

MATERIALS AND METHODS:

Source of Data: Blood samples received in the microbiology department at Jawaharlal Nehru Medical College, KAHER, Belagavi.
Study Design:One-year cross-sectional study
Study Period:1st January 2024 to 31st December 2024
Sample Size: Universal sample size
Sampling technique: Universal sampling technique
Inclusion Criteria: All blood samples from blood cultures received from Dr. Prabhakar Kore Hospital, from all age groups**.**
Exclusion Criteria: None

Study protocol:



1. **Data collection procedure:** Blood samples would be analyzed per the above procedure. The zone of inhibition for the specific drugs will be noted**.** They will be tabulated, and all the data will be analyzed to detect ESBL producers and the effective treatment against them.

Specimencollection:

1. Blood samples were received from the Department of Microbiology, JNMC, Belagavi.

Sample inoculation Technique

2. Blood samples were inoculated by MacConkey agar and Chocolate agar, followed by incubation for period of 18 to 24 hours at 37 °C.

Procedure:-

3. Blood sample is inoculated on the suitable culture media like Chocolate agar and MacConkey agar with the help of Inoculating loop and incubated at 37 °C aerobically for 18 to 24 hour

Observation:

4. **Colony count**: A microbial colony's visible cultural features on an agar plate are known as the morphology of the colony. The way the organisms grow on or on medium is highly significant when identifying bacteria. Using an agar plate, this activity will assist you in determining the colony morphology, or cultural traits, of a bacterium.

5. **Identification of isolates-** The usual colony features and accepted Biochemical tests were used to identify all bacterial isolates.

6. **Characteristic growth of Micro Organisms*:***

7. **On Chocolate Agar:**Small/Large 1-3mm, circular/ irregular, moist, flat/mucoid, colony seen. **On** 10.**MacConkey Agar:** Small/large, 1-3mm, circular/ irregular, pink, flat/mucoid, lactose fermentation colony or non-lactose fermentation colony is seen.

11.**Gram Stain:** Gram Positive & Gram Negative- organisms can seen

12. **IDENTIFICATION OF THE DIFFERENT ORGANISMS FROM THE ISOLATED COLONY BY STANDARD BIOCHEMICAL REACTIONS:**

1. Catalase Test: (+)Positive / (-)Negative
2. Coagulase Test: (+)Positive / (-)Negative
3. Oxidase Test: (+)Positive / (-)Negative
4. Motility Test: motile / non motile
5. Oxidative/Fermentative medium: Oxidative and fermentative
6. Nitrate reduction test: Positive / Negative
7. Mannitol motility medium: Fermented and motile
8. Triple sugar Iron Agar: A/A with gas and H2S not produced
9. Christensen's Urease media: Urea not hydrolyzed
10. MR-VP: Positive / Negative
11. Sugars test: Glucose, Mannitol, sucrose, lactose, Galactose, etc…..

Antibiotic Susceptibility Testing

Following the 2017 CLSI guidelines, antibiotic discs were categorized into A, B, C, and D groups. Subsequently, the sensitivity of all bacterial isolates and antibiotics was analyzed with the help of Kirby-Bauer disc diffusion method.

TABLE 1: Groups for Antibiotic Susceptibility Testing.

Group 'A'	**Group 'B'**	**Group 'C'**
Ampicillin – 10mcg	Amikacin - 30mcg	Azetronam - 30mcg
Gentamycin-10mcg	Amoxyclav - 30mcg	Ceftazidime - 30mcg
Tobramycin - 10 mcg	Cefipime - 30mcg	Tetracycline – 30mcg
	Pipperacillin/Tazobactam 100/10 mcg	Chloramphenicol – 30mcg
	Cefotaxime - 30mcg	**Group D**
	Levofloxacin - 5mcg	Colistin-10mcg
.	Imipenem - 10mcg	
	Meropenem - 10 mcg	
	Polymyxin-10mcg	

The CLSI criteria were followed to measure and interpret the Zone of Inhibition.

RESULTS:

The J N Medical College Microbiology Department receives blood samples from Dr. Prabhakar Kore Hospital and MRC Belagavi. The samples undergo culture, identification, and testing for antibiotic sensitivity patterns.

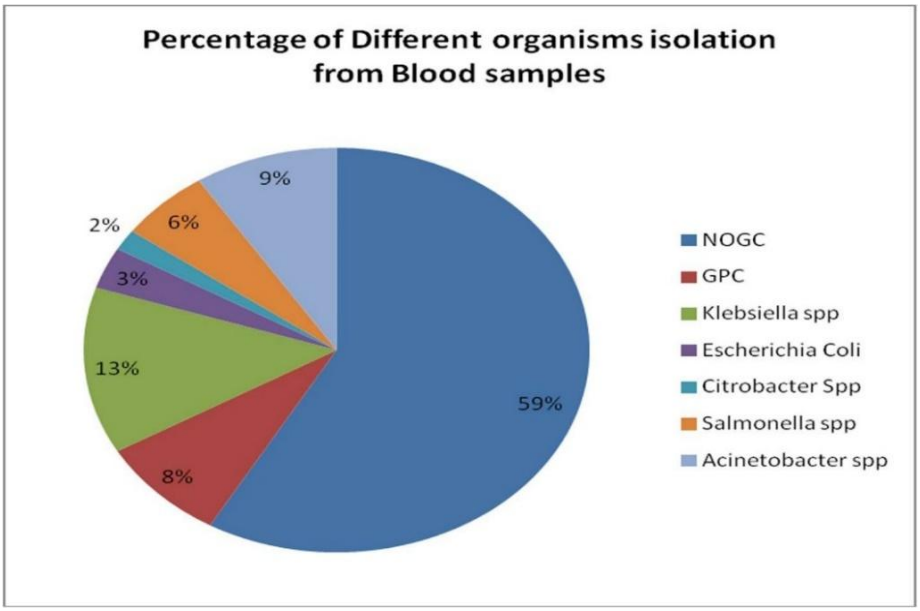
Table No. 2: Number of Organisms Isolated

8.	9. Total	10. Percentage
11. Total SSI Swab (Pus) Samples Received	12. 120	13. 100%
14. NOGC	15. 70	16. 58.33%
17. GPC	18. 10	19. 8.33%
20. Klebsiellaspp	21. 16	22. 13.33%
23. Escherichia Coli	24. 04	25. 3.33%
26. CitrobacterSpp	27. 02	28. 1.67%
29. Salmonella spp	30. 07	31. 5.83%
32. Acinetobacter spp	33. 11	34. 9.17%

Graph 1:Percentage of Different organisms isolated from Blood sample

A total of 120 samples were processed at this time. Of those 120 samples, 70 were found to have NOGC, 10 were found to have GPC, 16 were found to have *Klebsiellaspp*, 04 were found to have *Escherichia coli*, 02 were found to have *Citrobacterspp*, 07 were found to have *Salmonella spp*, 11 were found to have *Acinetobacter spp.*

Graph 2: Percentage of Different organisms isolated from Blood sample



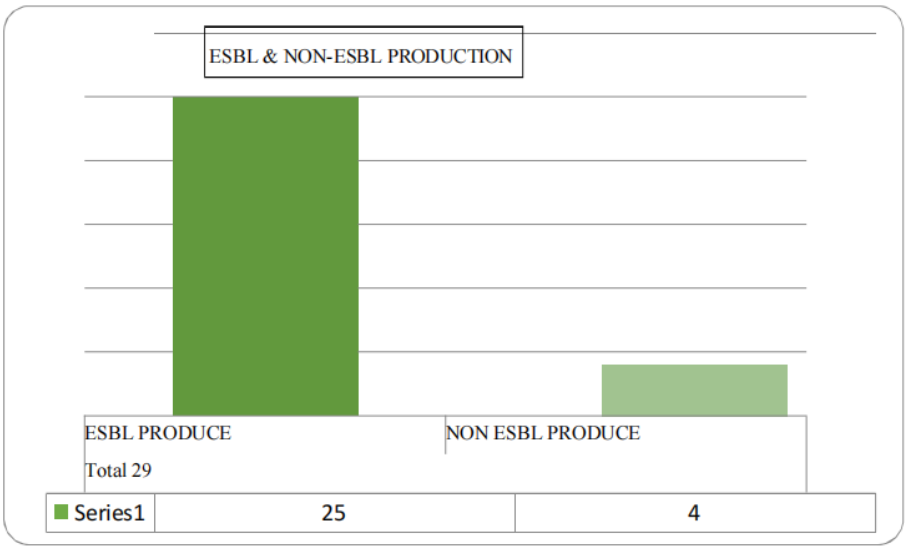
A total of 120 samples were processed at this time, and of those 120 samples, (59%) 70 were found to have NOGC, (08%) 10 were found to have GPC, (13%) 16 were found to have *Klebsiellaspp*, (03%) 04 found to have *Escherichia coli*, (02%) 02 were found to have *Citrobacterspp*, (06%) 07 were found to have *Salmonella spp*, (09%) 11 were found to have *Acinetobacter spp*

DISTRIBUTION OF ESBL & NON-ESBL PRODUCING BACTERIA

Among the twenty-nine (29) Enterobacteriaceae isolates processed, twenty-five were identified ESBL producers, while four are non-ESBL producers.

ORGANISMS ISOLATED	ESBL PRODUCERS	NON-ESBL PRODUCERS	TOTAL
Klebsiella Pneumoniae	35. 15	36. 01	37. 16
Escherichia Coli	38. 04	39. 00	40. 04
Salmonella spp	41. 04	42. 03	43. 07
CitrobacterSpp	44. 02	45. 00	46. 02
Total	47. 25	48. 04	49. 29

Graph 3:ESBL & NON-ESBL PRODUCTION



Distribution According to Clinical Diagnosis

Septic Shock	**05**	**12.5%**
Fever	**07**	**17.5**
Enteric Fever	**04**	**10%**
Pneumonia	**02**	**05%**
Dengue	**02**	**05%**
Others	**20**	**50%**

Others: Sinuses, Rheumatoid Heart Disease, Navicular fracture, pulmonary edema, cerebellar infarction, urosepsis with HTN, Hypoxia with Ischemic encephalopathy, HbSAg +ve with T2DM with HTN, etc.…

DISCUSSION:

A total of 120 samples were processed at this time, and of those 120 samples, (59%) 70 were found to have NOGC, (08%) 10 were found to have GPC, (13%) 16 were found to have *Klebsiellaspp*, (03%) 04 found to have *Escherichia coli*, (02%) 02 were found to have *Citrobacterspp*, (06%) 07 were found to have *Salmonella spp*, (09%) 11 were found to have *Acinetobacter spp*.

Twenty-nine (29) Enterobacteriaceae were processed in total at this time. Of those 29 Enterobacteriaceae isolated, 25 were ESBL producers, and 04 were non-ESBL producers. Infections primarily caused by Gram-negative bacteria are commonly treated with antibiotics such as cephalosporins, carbapenems, cephamycins, and aminoglycosides.

Every day, more and more ESBL beta-lactamases are developing and imparting resistance to these antibiotics. Prompt and efficient antibiotic therapy is required to guarantee the survival of patients suffering from Gram-negative bacteremia.

CONCLUSION:

It was looked at how 40 different Enterobacteriaceae types make the ESBL beta-lactamase enzyme. CitrobacterSpp, Salmonella Spp, Klebsiella pneumoniae and Escherichia coli can fight antibiotics in a big way by making this enzyme.Because of this, finding ESBL makers early and correctly will help lower the death and illness rates caused by using antibiotics without thinking and following strict hand hygiene rules.

The hospital should use a lot of medicines wisely and put in place the proper infection control measures.There were a lot of Klebsiella pneumoniae, ESBL-producing E. coli & Salmonella spp. Klebsiella pneumoniae & ESBL-producing E. coli are important signs for keeping an eye on AMR around the world, and the fact that they are found so often in India shows that there is an AMR risk there.

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