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PERFORMANCE OF IN-HOUSE ANTIBIOTIC DISC IN COMPARISON TO AUTOMATED SYSTEM VITEK-2 COMPACT SYSTEM FOR GRAM NEGATIVE ORGANISMS.

Microbiology

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ABSTRACT

Objectives: Commercially available antibiotic discs sometimes fail in CLSI defined quality control tests. This may be due to inferior quality control in discs manufacture process, low potency of antibiotic used or mismanagement in the supply chain, often resulting in rejection of supplies. This leads to long lag period in demand and supply of discs, especially in government set-up. Aim was to compare the antimicrobial susceptibility testing results of in-house prepared discs with the automated system VITEK-2 compact system in gram negative organisms.

Material and Methods: The study was conducted in Department of Microbiology, in tertiary hospital, New Delhi. Gram negative strains isolated at Pus seat were included in the study. In house antibiotic discs were prepared for gentamicin, ciprofloxacin, ceftriaxone, amikacin, meropenem, Imipenem and ampicillin using respective antibiotic powders and 6mm discs punched from Whatman filter paper no.1. Susceptibility testing was done using disc diffusion method and Vitek2 Compact. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Result: 120 gram negative bacilli were included in the study. Quality control results were within range except some minimum outliers. Maximum percent agreement between in-house discs and Vitek 2 compact system were ampicillin & carbapenam (90% &88.4%) antibiotics respectively. Taking Vitek2 as standard, very major errors 2% to 8% and major errors from 2% to 6.5% were seen.

Conclusion: In-house discs can be used as an alternative to commercially available antibiotic discs.

KEYWORDS

In- house antibiotic disc, VITEK 2 compact system & *Gram negative bacilli*

INTRODUCTION

Antibiotics are substances produced by a microorganism that inhibits the growth of other microorganisms or kill the organisms. They are sometime called Antibacterial or Antimicrobials.[1] The indiscriminate and irrational use of antibiotics and other reasons have led to a global challenge of antimicrobial resistance.[2] Commercially available antibiotic discs sometimes fail in CLSI defined quality control tests. This may be due to inferior quality control in discs manufacture process, low potency of antibiotic used or mismanagement in the supply chain, often resulting in rejection of supplies. This leads to long lag period in demand and supply of discs, especially in government set-up. Rapid bacterial identification and susceptibility testing improve patient therapy and outcome, decreases emergence of resistance [3,4] There is a need to provide rapid, efficient and accurate system for identification and antimicrobial susceptibility testing of pathogens. In this regard the automated identification/AST systems aid in rapid diagnosis/treatment of bacterial pathogens. Antimicrobial discs should be manufactured under strict compliance to standard operative procedures; otherwise, quality and performance standards will be compromised.[5] The locally prepared antibiotic discs are readily available for use and are also cost effective. They seem to be more potent because they are prepared at the point of use, not exposed to excessive heat during transportation to the various retail outlets.[6] The standard procedures may not be met in the developing countries compared to developed countries.[7] Automated identification/AST systems can aid in rapid diagnosis of bacterial pathogens. The VITEK-2 compact is an automated system for identification and antibiotic susceptibility testing. VITEK-2 susceptibility test results are expressed as Minimum Inhibitory Concentration (MIC) values and interpreted as susceptible, intermediate or resistant by reference to a CLSI.[8] They seem to be more potent because they are prepared at the point of use, not exposed to excessive heat during transportation to the various retail outlets. In-house prepared antibiotics are preferred because, it saves time, money, and cost of transportation and delay when compared to the commercially prepared disc which is not readily available.[8,9] This study was carried out to compare the antimicrobial susceptibility testing results of in-house prepared discs with the automated system VITEK-2 compact system in gram negative organisms.

MATERIAL AND METHODS

A cross sectional Prospective, observational six months (April to December 2019) study was conducted in Department of Microbiology, in a government tertiary hospital, New Delhi. 120 gram negative strains were included in the study in which 75 *Enterobacteriaceae* strains (*Escherichia coli*- 50 strains, *Klebsiella pneumoniae*- 17 strains & *Proteus species*- 18 strains) and 45 Nil-fermenting Gram-negative strains (*P. aeruginosa*-11 strains, *Acinetobacter species*- 34 strains) isolated from pus seat were included in present study.

In house antibiotic discs were prepared for gentamicin, ciprofloxacin, ceftriaxone, amikacin, meropenem, Imipenem and ampicillin using respective antibiotic powders and 6mm discs punched from Whatman filter paper no.1. Susceptibility testing was done using disc diffusion method and Vitek2 Compact. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.[8]

- **Materials used**
- Antibiotic powder of gentamicin, ciprofloxacin, ceftriaxone, amikacin, meropenem, Imipenem and ampicillin.
- Distilled water
- Whatmann filter paper no 1
- 0.5 McFarland standard
- Tuberculin syring
- Sterile Petri dish Punching machine

- **Quality control strains used**
- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 25923
- *Pseudomonas aeruginosa* ATCC 27853

- **Culture media used**
- Mueller-Hinton Agar
- Peptone water

1) Preparation of filter paper discs

- For the purpose of product in of antibiotics discs, whatman filter

paper No.1 was used.
- To facilitate the identification of the discs, code names and the concentration of the drug in each disc were printed on the filter paper.
- Using an ordinary office hole punching machine, holes of approximately 6mm diameter were punched.
- Precautions were taken to avoid overlapping of holes.
- Since the paper discs have a tendency to curl after punching, they were straightened by keeping them onto a flat surface and applying pressure on them.
- The discs were then autoclaved at 15lbs pressure for 30 minutes.

2) Preparation of antibiotics stock solution
- Standard antibiotic powders were obtained commercially.
- Known weight of antibiotic powder was dissolved in sterile distilled water to obtain the stock solution.
- The stock solution was diluted at the time of disc preparation to obtain the working solution.
- A paper disc of 6mm diameter can absorb 0.02ml or 20µl of solutions. The concentrations of antibiotic solutions were expressed in µg/µl.

3) Impregnation of the discs
- Sterile discs were placed in petri dishes approximately 5mm apart.
- Using a mechanical pipette/ tuberculin syring, a fixed volume of 20µl was loaded on each disc one by one, taking precautions that the tip was in slight contact with the disc.

4) Drying and Storage
- Without covering the petri dishes, the discs were allowed to dry in a clean incubator at 37°C for 4 hours.
- After drying, discs were placed in small sterile air-tight labeled containers.
- The discs were stored in a freezer at -20°C.
- The discs were removed from the freezer 1 to 2 hours prior to usage so that the amount of condensation that may occur when warm room air reaches the cold containers.

5) Standardization of antibiotic disc:
- The prepared antibiotics were tested for their efficacy using the Kirby-Bauer disc diffusion method and were checked if the diameter of the zone of inhibition was between the ranges for sensitivity of the organisms.
- The test organisms used were Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 7553 standard strains.
- The culture used were sub cultured a day before the sensitivity testing as 24 hours young cultures are required for accurate results.
- The inoculums were prepared from the cultures and were matched for turbidity with 0.5 Mcfarland solutions.
- The prepared antibiotic discs were placed on the inoculated agar plate along with the commercially available discs for comparison of the efficacy of the prepared discs.
- The plates were then incubated at 37 °C overnight.
- After incubation, the zone of inhibition was measured for each of the antibiotic disc and was seen if they were within the sensitivity range of the organism.

Antimicrobial susceptibility testing of strains:

Antimicrobial Susceptibility testing: It was determined by the Kirby-Bauer disc diffusion method on Muller Hinton agar, and interpreted according to the recommendations of the Clinical Laboratory Standards Institute. The antibiotic disc was applied using a sterile forceps and sufficiently separated from each other in other to prevent overlapping of the zone of inhibition. The plates were incubated at 370C for 18-24hours. The zone of inhibition was measured with a meter rule and it was compared with the CLSI interpretive guide for antimicrobial susceptibility testing. The Vitek-2 compact system is an automated system for identification and antibiotic susceptibility testing. Vitek susceptibility test results are expressed as Minimum Inhibitory Concentration (MIC) values and interpreted as susceptible, intermediate or resistant by reference to a CLSI. All isolates were tested with gram negative susceptibility cards (GN AST: 280 & 281).The inoculum prepared were processed on to the automated system VITEK 2 compact for gram negative organisms.

STATISTICALANALYSIS:

The Results obtained were expressed as percentages. Percent agreement between results obtained by VITEK 2 and disc diffusion was calculated. Very Major Errors (Disk diffusion sensitive & VITEK 2 resistant) and Major errors (Disc resistant & VITEK sensitive) were also calculated.

RESULT

Both methods were comparable with ATCC control strains and the quality control test showed correct results. In present study total 120 strains of gram negative bacilli were isolated. Patient from whom gram negative isolates were recovered included 80 (61.5%) males and 50 (38.5%) females. Out of 120 strains, in which 75 strains were *Enterobacteriaceae* and 45 strains were Nil- fermenting. 75 gram negative strains were *Enterobacteriaceae* which included *Klebsiella pneumoniae* (17), *Escherichia coli* (50) *& Proteus spp.* (8) while, 45 Nil-fermenting gram-negative strains including *P. aeruginosa* (11) and Acinetobacter *species* (34) were obtained from various clinical samples (Pus aspirate, ascitic fluid, peritoneal fluid, wound swab & pleural fluid) from pus seat. The antibiotic susceptibility of all strains was performed from in house disc by Kirby-Bauer disc diffusion method. Antimicrobial susceptibility of all strains were confirmed by VITEK 2 compact (GN AST: 280 & 281 card) system.

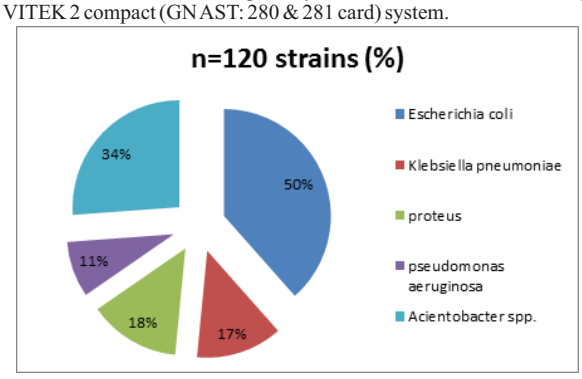

Fig 1: Depicted the prevalence of Gram negative strains isolated from clinical samples.

Maximum percent agreement between in-house discs and Vitek 2 compact system for Enterobacteriaceae were ampicillin & carbapenam (90% &88.4%) antibiotics respectively. For nil-fermenter it was 83%, 89%, 92%and 94% for amikacin, gentamicin, meropenem and Imipenem, respectively. (Table 1)

Table 1: Comparison of Percentage agreement of various antibiotics disc versus VITEK 2 compact system.

a. GENTAMICIN				
DISC	VITEK			
		R	IS	S
	R	25	0	05
	IS	0	01	0
	S	03	01	41

% AGREEMENT : 89.4%

b. CIPROFLOXACIN				
DISC	VITEK			
		R	IS	S
	R	33	0	04
	IS	0	0	02
	S	04	02	31

% AGREEMENT: 85.4%

c .CEFTRIAXONE				
DISC	VITEK			
		R	IS	S
	R	52	01	02
	IS	01	0	0
	S	05	01	13

%AGREEMENT – 87%

d .AMIKACIN				
DISC	VITEK			
		R	IS	S
	R	25	01	05
	IS	02	02	0
	S	06	02	32

% AGREEMENT- 77.4%

e IMIPENEM				
VITEK				
DISC		R	IS	S
	R	24	01	02
	IS	01	01	02
	S	03	0	41

% AGREEMENT- 88%

f AMPICILLIN				
VITEK				
DISC		R	IS	S
	R	45	01	02
	IS	01	0	01
	S	02	01	22

% AGREEMENT- 90%

R: Resistant, S: Sensitive, IS: Intermediate sensitive.

Table 2: Different antibiotics showing Very major error and Major error (%).

ANTIBIOTICS NAME	VERY MAJOR ERROR (%)	MAJOR ERROR (%)
GENTAMICIN	4 %	6.6%
CIPROFLOXACIN	5.3 %	5.3%
CEFTRIAXONE	6.6%	2.6%
AMIKACIN	8%	6.6%
IMIPENEM	4%	2.6%
MEROPENEM	4%	4%
AMPICILLIN	2.6%	2.6%

Taking Vitek2 as standard, very major errors 2% to 8% and major errors (ME) from 2% to 6.5% were seen in Enterobacteriaceae. While, very major errors (VME) ranged from 0% to 4.4% and major errors from 0% to 6.6% were noticed in Nil-fermenters. (Table 2)

DISCUSSION

Antibiotic sensitivity testing is an important part in the treatment of infections. The most commonly used method is the Kirby-Bauer disc diffusion method. Commercially available antibiotic discs are expensive and therefore not feasible for use in laboratories in developing countries. Therefore a low cost alternative will be the preparation of antibiotic discs locally.[10] The efficacy of the in house prepared antibiotic discs was studied by comparing them with automated system VITEK 2 compact using the standard strains of different of bacteria. They were compared for their zone of inhibition values. A key goal of this study was to assess whether the disk diffusion and VITEK 2 methods gave accurate susceptibility test results for a collection of isolates of *gram negative organisms.* The study recorded a susceptibility profile of 66.7% to 100% for in-house prepared antibiotic discs while the range for VITEK 2 compact system was 53.3% to 86.7%. The in house prepared antibiotic discs were equally potent than commercial discs.[11] Thus may be due to high temperature that these commercially prepared antibiotics are exposed to during transportation from the manufacturer to the end users, time of production, storage facility.[12] 63% gram negative isolates were tested including member of family *Enterobacteriaceae* including *Klebsiella pneumoniae* and *Escherichia coli* while 37% non fermenter have *Pseudomonas* spp. and *Acinetobacter spp*. categorical agreement between VITEK 2 compact system and Kirby- bauer disc diffusion method was 95.7% which included VME (2-8%) and ME (2-6%) as compared to study done in central government hospital, Delhi by Duggal etal. (VME 1.23% and ME 1.23%) [13]

Drawbacks in our study are that comparison of antimicrobial susceptibility has been done by MIC in automated VITEK-2 compact system with zone diameters of disc diffusion method, and concordance of isolates is compared only up to genus level in some instances.

CONCLUSION

Resistance to antibiotics is a major concern worldwide. Antibiotic resistance has caused a lot of morbidity and mortality all over the world. In present study in house antibiotics have been prepared and also standardized by comparing their efficacy with the VITEK 2 AST compact system. They were found to be almost equally effective when compared with VITEK 2 compact system. So,In-house discs can be used as an alternative to commercially available antibiotic discs.

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