ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 14, Issue 10, 2021

Online - 2455-3891
Print - 0974-2441

Research Article

CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF NON LACTOSE FERMENTING GRAM-NEGATIVE BACTERIAL ISOLATES IN A TERTIARY CARE TEACHING HOSPITAL OF CENTRAL INDIA

ABHISHEK MEHTA[1]*, MANISH KR DIWAKAR[2]

[1]Department of Microbiology, Government Medical College, Datia, Madhya Pradesh, India. [2]Department of Microbiology, State Medical College, Shahjahanpur, Uttar Pradesh, India. Email: abhishekmehta623@gmail.com

Received: 30 June 2021, Revised and Accepted: 28 August 2021

ABSTRACT

Objectives: The non-lactose fermenting Gram-negative bacilli (NLF-GNB) are notorious pathogens reportedly acquiring multiple drug resistance alarmingly and emerging as a public health threat globally. This study was conducted to isolate and identify these pathogens from clinical samples received routinely in our Bacteriology Laboratory and to analyze their antibiotic susceptibility patterns.

Methods: In this cross-sectional study, the first 100 NLF-GNB strains isolated consecutively from 1218 clinical samples were included through convenience sampling. The samples were processed using standard microbiological techniques.

Results: The most common isolate was *Pseudomonas aeruginosa* followed by *Acinetobacter* spp, *Proteus* spp, *Shigella* spp, *Salmonella typhi, Providencia* spp., and *Morganella* spp. *P. aeruginosa* and *Acinetobacter* spp. isolates were found to exhibit high susceptibility toward Colistin and Imipenem. *Proteus* spp. exhibited high sensitivity toward Imipenem, Aminoglycosides, Ceftazidime, and Cefepime. All *Providencia* isolates were susceptible to Amikacin, Cefepime, and Ceftriaxone. The only isolate of *Morganella* spp. was found to be susceptible to Amikacin, Cefepime, Ceftazidime, Piperacillin tazobactam, Ciprofloxacin, Imipenem, and Aztreonam. *Shigella* isolates exhibited very high susceptibility toward Imipenem followed by Gentamicin and Ceftazidime. All the isolates of *S. typhi* exhibited susceptibility toward Imipenem, Piperacillin tazobactam, Ceftazidime, Ceftriaxone, Cefoperazone sulbactam, and Chloramphenicol. 24% of test isolates were found to be Multidrug resistant.

Conclusion: Antimicrobial surveillance is needed to implement appropriate timely interventions to restrict the spread of multidrug-resistant clones. Strict infection prevention and control practices, with judicious antibiotic prescription policy, may help in tackling this problem by obviating the selection pressure.

Keywords: Non-lactose fermenting Gram-negative bacilli, Antimicrobial susceptibility, Antibiotic resistance, Samples, Isolates, Multidrug resistance.

 DOI: http://dx.doi.org/10.22159/ajpcr.2021v14i10.42822. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Aerobic non-fermenting Gram-negative bacilli (NFGNB) are a taxonomically diverse group of bacteria that are either not capable of utilizing carbohydrates as an energy source or degrading them via oxidative pathway [1-3].

NFGNB (*Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Stenotrophomonas*, etc.) constitute about 15% of all clinical bacterial isolates. NFGNBs are emerging as important health-care-associated pathogens in the current scenario. Hospital strains are found to exhibit multidrug resistance (MDR). They have been incriminated in infections, such as septicemia, meningitis, pneumonia, urinary tract infections, and surgical site infections. NFGNBs are innately resistant to many antibiotics and are known to produce extended-spectrum ß-lactamases (ESBL) and Metallo ß-lactamases [4-6].

Apart from NFGNB, there is another group of non-lactose fermenters capable of utilizing glucose (*Proteus*, *Salmonella*, *Shigella*, *Morganella*, *Providencia*, etc.). These NLF-GNB are Notorious pathogens reportedly acquiring multiple drug resistance alarmingly and emerging as a public health threat.

MDR has been emerging rapidly and consistently in these groups of pathogens driven by selection pressure due to inappropriate irrational drug therapy. This has resulted in treatment failures leading to an extended hospital stay, health complications, and a significant rise in morbidity and mortality. The study of antibiotic susceptibility patterns of isolates is imperative in formulating a strategy for prompt and appropriate therapy and also plays a crucial role in the prevention and control of the disease. Continual consistent surveillance and monitoring of local antimicrobial resistance trends is a prerequisite for implementing rational measures and updating the therapeutic guidelines [4,6-8].

There are very few studies from India wherein the various NLF-GNBs, isolated from patients' samples, have been identified and their clinical significance assessed. Hence, this cross-sectional study was undertaken to identify the various non-lactose fermenters isolated from the patients attending our hospital to assess their clinical significance and antimicrobial susceptibility pattern.

METHODS

This cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Datia (MP), India, after obtaining clearance from Institutional Ethics Committee.

Study period and clinical samples

Samples such as blood, urine, sputum, wound swabs, pus, stool, other body fluids, ear swabs, throat swabs, and nasal swabs obtained from patients admitted or attending OPDs of various clinical departments in GMC, Datia, submitted to the Department of Microbiology for routine diagnostic workup.

Sample size

Through convenience sampling, first 100 non-lactose fermenting Gram-negative bacterial strains isolated consecutively from the clinical

samples processed as per the standard protocol in the duration between October 1, 2018, to April 30, 2020, whichever is earlier were included in the study.

The NLF-GNB isolates found to be contaminants or commensals concerning for the respective samples were excluded from the study. For e.g., *Pseudomonas aeruginosa* or *Proteus* spp. if isolated from stool culture were not included in the study, being a part of commensal flora of gastrointestinal tract.

Isolation and identification of bacterial isolates were done using standard microbiological techniques. The antimicrobial susceptibility test of all the test isolates was performed by the Kirby–Bauer Standard disc diffusion method and results were interpreted according to the CLSI 2018 guidelines [9-11].

RESULTS AND DISCUSSION

Through convenience sampling, first 100 non-lactose fermenting Gram-negative bacterial strains were isolated consecutively from 1218 clinical samples, starting from October 1, 2018. The study target was achieved till January 31, 2020 (16 months).

The majority of samples yielding NLF-GNB test isolates belonged to the patients in the age group of 40–60 years (42%) followed by the patients in the age group of 20–39 years (34%).

Similar findings were observed in the study conducted by Grewal *et al.* 2020, Benachinmardi *et al.* 2020, Reddy *et al.* 2019, Sajjad *et al.* 2017, and Bohra *et al.* 2017. However, Akbar *et al.* 2014 in a similar study conducted in Peshawar, Pakistan, found that most of the test isolates were contributed by the females and patients in the 11–30 years age group [1,8,12-14].

As evident in Fig. 1, more than half of the NLF test isolates were obtained from pus and urine. This finding is in concordance with other similar studies conducted by Reddy *et al.* 2019, Bhargava *et al.* 2015, Akbar *et al.* 2014, Malini *et al.* 2009, and Grewal *et al.* 2020. But in the majority of studies conducted in the recent past, the test isolates were derived predominantly from pus and respiratory samples [1,7,12,15,16].

As evident in Fig. 2, the most common test organism isolated was *P. aeruginosa* followed by *Acinetobacter* spp. This finding is as per the similar studies by Upgade *et al.* 2012, Nautiyal *et al.* 2014, Gore and Pai 2015, Bhargava *et al.* 2015, Kamalraj *et al.* 2015, Mahajan *et al.* 2016, Sajjad *et al.* 2017, Gunasekar *et al.* 2018, Nazir *et al.* 2019, Reddy *et al.* 2019, Benachinmardi *et al.* 2020, Grewal *et al.* 2020, and Juyal *et al.* 2020 [1,2,5,7,8,12,13,17-21].

But unlike our study, in few similar studies conducted by Sharma and Pant, 2017, Shah and Vaghela, 2018, and Paul and Borah, 2020, *Acinetobacter* spp. was the most common isolate followed by *P. aeruginosa* [4,22,23].

Out of the 100 samples yielding the test isolates, polymicrobial growth was seen in 22 samples where the NLF-GNB were isolated along with other organisms. None of the samples yielded more than one NLF isolate.

The overall isolation rate of NLF isolates from the total 1218 clinical samples processed came out to be 8.2%. *P. aeruginosa* (4%) exhibited the highest isolation rate followed by *Acinetobacter* (2%). The sample-wise and organism-wise isolation rates are depicted in Tables 1 and 2.

As evident in Table 3, *P. aeruginosa* was more common in pus, ear swabs, and urine contributing to 80% of its isolates, while less common in blood, body fluids, sputum, and throat swabs. This finding is in concordance with other similar studies conducted by Reddy *et al.* 2019, Bhargava *et al.* 2015, Akbar *et al.* 2014, Malini *et al.* 2009, and Grewal *et al.* 2020 [1,7,12,15,16].

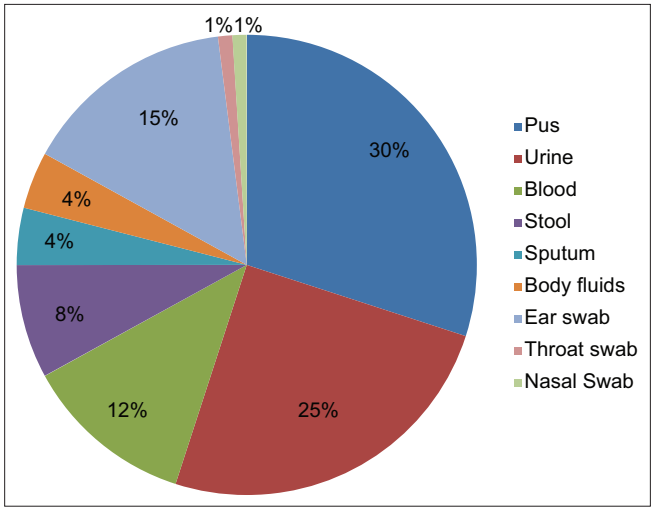

Fig. 1: Sample wise distribution of NLF isolates

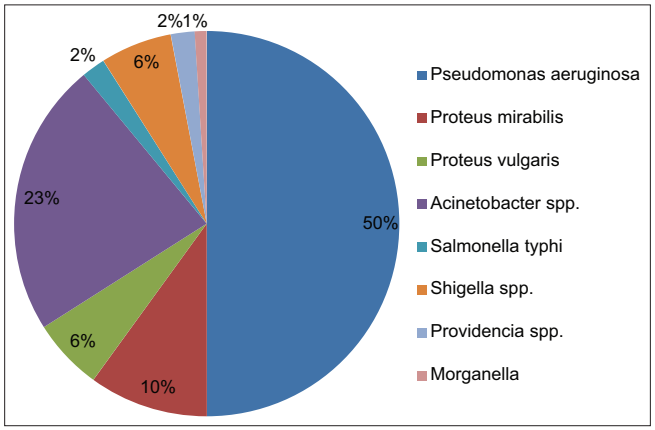

Fig. 2: Organism-wise distribution of non-lactose fermenting isolates

Table 1: Sample-wise isolation rate of test isolates

S. No.	Specimens	Number of samples	No. of NLF isolates	% isolation
1.	Pus	450	30	6.67
2.	Urine	438	25	6
3.	Blood	114	13	11.4
4.	Stool	66	08	12
5.	Sputum	56	04	7
6.	Body fluids	46	04	9
7.	Throat swab	28	01	3.6
8.	Nasal Swab	20	01	5
	Total	1218	100	8.2

Table 2: Organism-wise isolation rate of non lactose fermenting isolates

S. No.	Organism	No. of isolates	% isolation
1.	*Pseudomonas aeruginosa*	50	4
2.	*Proteus mirabilis*	10	0.8
3.	*Proteus vulgaris*	05	0.4
4.	*Acinetobacter* spp.	23	2
5.	*Salmonella typhi*	03	0.25
6.	*Shigella* sp	06	0.5
7.	*Providencia* spp.	02	0.16
8.	*Morganella* spp.	01	0.08

Table 3: Sample-wise distribution of non-lactose fermenting isolates

S. No.	Non-lactose fermenting Isolates	Samples									
		Pus	Urine	Blood	Stool	Sputum	Ear swab	Body fluids	Throat swab	Nasal Swab	Total
1.	*Pseudomonas aeruginosa*	18	9	4	-	2	13	3	1	-	50
2.	*Proteus mirabilis*	4	5	-	-	-	1	-	-	-	10
3.	*Proteus vulgaris*	1	3	1	-	-	-	-	-	-	05
4.	*Acinetobacter* spp.	7	6	6	-	1	1	1	-	1	23
5.	*Salmonella typhi*	-	-	1	2	-	-	-	-	-	03
6.	*Shigella* spp.	-	-	-	6	-	-	-	-	-	06
7.	*Providencia* spp.	-	1	1	-	-	-	-	-	-	02
8.	*Morganella* spp.	-	1	-	-	-	-	-	-	-	01
	Total	30	25	13	08	03	15	04	01	01	100

In the present study, *Acinetobacter* spp. was found to be more common in pus, blood, and urine contributing to 83% of its isolates while less common in body fluids, sputum, ear swabs, and throat swabs. Similar findings were reported by Grewal *et al*. 2020, Reddy *et al*. 2019, and Kamalraj *et al*. 2015 [1,5,12] But in some studies by Malini *et al*. 2009, De Francesco *et al*. 2013, Benachinmardi *et al*. 2020, Sharma and Pant 2017, the organism was predominantly isolated from respiratory samples [4,8,16,24].

In the present study, most of the isolates of *Proteus* spp. (87%) were obtained from pus and urine like the other similar studies by Sharma and Pant, 2017, Wang *et al*. 2014, and Leulmi *et al*. 2014 [4,25,26] However, Bahashwan 2013 found that majority of *Proteus* spp. isolates were derived from sputum and wound swabs [27].

Out of three isolates of *Salmonella typhi*, two were obtained from stool culture and one from blood culture.

Shigella spp. being an enteropathogen, fecal specimens are the most preferred samples for culture. In our study too, all the six *Shigella* spp. isolates were obtained from stool culture as in the majority of similar studies [28-33].

Out of two isolates of *Providencia* spp., one each was isolated from urine and blood cultures. In the study period, we reported only one isolate of *Morganella* spp. from a urine sample.

In a study by Leulmi *et al*. 2014, *Providencia* spp. was mainly isolated from pus and urine cultures [26]. Liu *et al*. 2020, isolated *Providencia* spp. mainly from sputum and wound swabs [34].

In similar studies, *Morganella* spp. was predominantly isolated from pus, urine, and blood by Akbar *et al*. 2014, from pus and urine by Leulmi *et al*. 2014, and from urine by Sharma and Pant 2017 [4,15,26].

As evident in Fig. 3, the highest number of test isolates were obtained from clinical specimens from the ENT department followed by the Surgery, Pediatric, and Medicine departments. 60% of the NLF test isolates were obtained from IPD patients and 40% from OPD patients, while an opposite trend was seen in the samples from ENT department, wherein contribution from OPD patients (83%) far exceeded the IPD patients. On the other hand in the case of the Pediatric department, only 2 out of 18 isolates were obtained from OPD samples, with Pediatric ICUs (PICU and SNCU) contributing more than 60% of the test isolates.

The antibiotic susceptibility pattern of NLF test isolates is depicted in Table 4. In our study, *P. aeruginosa* isolates were found to exhibit high susceptibility toward Colistin and Imipenem and very less susceptibility toward Ciprofloxacin, Aztreonam, and Co-trimoxazole. Similar findings were reported by Shah and Vaghela, Sharma and Pant, and Bhargava *et al*. [4,7,22].

Grewal *et al*. reported high resistance toward Aminoglycosides, Amoxycillin clavulanate, Fluoroquinolones, Aztreonam, and third and fourth generation Cephalosporins [1].

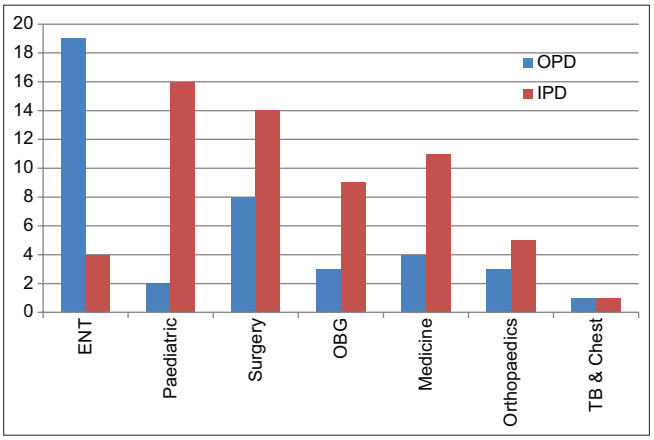

Fig. 3: Department-wise distribution of test isolates

Kamalraj *et al*. reported high susceptibility toward Imipenem and high resistance toward third-generation Cephalosporins (3GC) and Ciprofloxacin. They reported 38.3% and 42.3% ESBL strains, respectively [5].

Kamalraj *et al*., Nagaveni *et al*., and Anuradha *et al*. reported 11.6%, 24%, and 28 % of MBL producing strains of *P. aeruginosa,* respectively [5,35,36].

Malini *et al*. and Gore and Pai reported high susceptibility toward Imipenem, Ticarcillin, Amikacin, and Cefoperazone and least toward Co-trimoxazole [2,16].

Juyal *et al*. and Benachinmardi *et al*. reported high sensitivity toward Amikacin, Imipenem, Piperacillin tazobactam, and Ticarcillin clavulanate and least sensitivity toward Co-trimoxazole [6,8].

In our study, *Acinetobacter* spp. isolates were found to exhibit high susceptibility toward Colistin and Imipenem and very less susceptibility toward Ciprofloxacin, Cefepime, Ceftriaxone, Aztreonam, Amoxycillin clavulanate, Gentamycin, Tobramycin, and Co-trimoxazole.

Malini *et al*. reported high sensitivity toward Imipenem and Piperacillin and low susceptibility toward Ciprofloxacin, Co-trimoxazole, and third and fourth generation Cephalosporins [16]. Nautiyal *et al*. reported high sensitivity toward Imipenem and Piperacillin tazobactam and low sensitivity toward Aminoglycosides, Fluoroquinolones, 3GC, and Co-trimoxazole [18].

Grewal *et al*. reported high sensitivity toward Imipenem and Cefoperazone sulbactam and low sensitivity toward Polymixin group, Ureidopenicillin group, Amoxycillin clavulanate, Aminoglycosides, Fluoroquinolones, Aztreonam, and third and fourth generation Cephalosporins [1].

Table 4: Antibiotic susceptibility pattern of test isolates

S. No	Antibiotics	No. of susceptible isolates n (%)						
		Pseudomonas aeruginosa	***Proteus mirabilis Proteus vulgaris***	***Acinetobacter* spp.**	***Salmonella typhi***	***Shigella* sp.**	***Providencia* sp.**	***Morganella* sp.**
1.	Amikacin (30)	30 (60)	13 (86.67)	13 (56.5)	2 (66.7)	4 (66.7)	2 (100)	1
2.	Gentamicin (10)	25 (50)	13 (86.67)	10 (43.5)	1 (33)	5 (83)	0	0
3.	Cefepime (30)	30 (60)	12 (80)	9 (39)	2 (66.7)	2 (33)	2 (100)	1
4.	Ceftazidime (30)	32 (64)	11 (73.3)	11 (48)	3 (100)	5 (83)	1 (50)	1
5.	Ceftriaxone (30)	24 (48)	10 (66.67)	10 (43.5)	3 (100)	4 (66.7)	2 (100)	0
6.	Cefoperazone sulbactum	23 (46)	6 (40)	11 (48)	3 (100)	5 (83)	1 (50)	0
7.	Amoxycillin clavulanate	27 (54)	7 (46.67)	10 (43.5)	2 (66.7)	4 (66.7)	0	0
8.	Piperacillin tazobactam	34 (68)	7 (46.67)	12 (52)	3 (100)	5 (83)	0	1
9.	Ciprofloxacin (5)	23 (46)	7 (46.67)	7 (30)	2 (66.7)	5 (83)	1 (50)	1
10.	Trimethoprim sulfamethoxazole	19 (38)	5 (33.3)	6 (26)	2 (66.7)	2 (33)	1 (50)	0
11.	Imipenem (10)	42 (84)	15 (100)	19 (82.6)	3 (100)	6 (100)	1 (50)	1
12.	Aztreonam	13 (26)	-	3 (13)	-	-	0	1
13.	Colistin	50 (100)	-	22 (95.6)	-	-	-	0
14.	Chloramphenicol	-	8 (53.3)	-	3 (100)	3 (50)	0	0
15.	Tetracyclin	-	-	-	-	2 (33)	0	-
16.	Tobramycin	28 (56)	12 (80)	10 (43.5)	-	-	1 (50)	-

A very high level of resistance to different groups of antibiotics was reported by Benachinmardi *et al*., Juyal *et al*., Gupta *et al.,* and Hodiwala *et al*., but the strains were comparatively susceptible to Imipenem [6,18,37,38].

Uma *et al*. 2009 and Anil *et al*. 2011 reported 70.9% and 21% of MBL producing strains of *Acinetobacter baumannii*, respectively [39,40]. Kamalraj *et al*. and Sinha *et al*. reported 25% and 28 % of ESBL strains, respectively [5,41].

A. baumannii is gaining more importance as a nosocomial notorious pathogen due to its potential to form a biofilm, which accounts for its outstanding antibiotic resistance and high virulence.

Proteus spp. in the present study exhibited high sensitivity towards Imipenem, Tobramycin, Amikacin, Gentamicin, Ceftazidime, and Cefepime and less sensitivity toward Cefoperazone sulbactam, Amoxycillin clavulanate, Piperacillin tazobactam, Ciprofloxacin, and Co-trimoxazole.

Proteus mirabilis strains are generally found to be more susceptible to antimicrobials than *Proteus vulgaris* and other *Proteus* species. *P. mirabilis* has got intrinsic resistance to nitrofurantoin and tetracycline which could be used as an identification marker. However, it is generally susceptible to the Carbapenems, Penicillins, Aminoglycosides, and Co-trimoxazole [42].

Bahashwan 2013 reported high sensitivity of *Proteus* spp. isolates toward Imipenem followed by Amikacin, Cefoxitin, Aztreonam, and Piperacillin. The other antibiotics (Amoxycillin clavulanate, Gentamicin, Ceftazidime, Ciprofloxacin, Cephalothin, Cefpiramide, and Co-trimoxazole) exhibited <40% sensitivity [27].

Feglo *et al*. 2010 reported very high levels of resistance in *Proteus* spp. isolates against Ampicillin, Co-trimoxazole, Tetracycline, and Chloramphenicol [43].

Mirzaei *et al*. reported high sensitivity in urinary isolates of *P. mirabilis* toward Amoxicillin-clavulanate, Carbapenems, 3GC, Aztreonam, Tobramycin, and Fluoroquinolones and low sensitivity toward Cotrimoxazole and Amoxicillin. They found all isolates exhibiting the capability of biofilm formation with 72% of strains being strong biofilm producers. 24% of isolates were sensitive to all antibiotics tested, while one isolate was pan resistant. 14.5% of the isolates were MDR strains and 10% were ESBL producers. Most of the ESBL producers were MDR strains [44].

Swenson *et al*. 1999 have shown that all *P. vulgaris* and *Proteus penneri* strains have got the capability of producing inducible β-lactamases which could hydrolyze primary and extended-spectrum penicillins and cephalosporins. This calls for the need for monitoring the drug susceptibility of *Proteus* isolates [45].

Since we have only one *Morganella* spp. and two *Providencia* spp. isolated in the present study, any statistically significant inference could not be drawn from the existing study data regarding antimicrobial resistance.

The only isolate of *Morganella* spp. was found to be susceptible to Amikacin, Cefepime, Ceftazidime, Piperacillin tazobactam, Ciprofloxacin, Imipenem, and Aztreonam and resistant to Gentamicin, Ceftriaxone, Cefoperazone sulbactam, Amoxycillin clavulanate, Trimethoprim-sulfamethoxazole, Colisin, and Chloramphenicol.

All *Providencia* isolates were found to be susceptible toward Imipenem, Amikacin, Cefepime, and Ceftriaxone, while none toward Gentamycin, Amoxycillin clavulanate, Piperacillin tazobactam, Aztreonam, Chloramphenicol, and Tetracyclin.

Lieu *et al*. reported high susceptibility of *Providencia stuarti* isolates toward Imipenem, Fluoroquinolones, Amikacin, and Cefepime. All test isolates were resistant to Ampicillin sulbactam, Amoxicillin clavulanate, Ticarcillin clavulanate, Piperacillin tazobactam, Aztreonam, and 3GC. All 76 isolates were found to be ESBL producers out of which 92% exhibited multiple drug resistance [34].

In hospital settings, high levels of Ciprofloxacin resistance have been reported frequently for the *Proteus* and *Providencia* spp. isolates due to rampant usage of the same. Fass *et al*. 1995 reported decreasing susceptibility of *P. stuartii* to ciprofloxacin from 100% to 46% over a 6 year period [46].

P. penneri is generally found to be more resistant to penicillins than *P. vulgaris*, and its susceptibility pattern resembles more with *Morganella morganii* than with other *Proteus* spp.

M. morganii and *P. penneri* are generally susceptible to cefoxitin, 3GC,4GC, aztreonam, aminoglycosides, ciprofloxacin, and imipenem and resistant to cefazolin, 1GC, 2GC, Cefoperazone, amino, and ureidopenicillins.

Leulmi *et al*. reported high sensitivity of *Proteus*, *Providencia*, and *Morganella* spp. isolates toward cefoxitin, cefotaxime, imipenem, amikacin, and ciprofloxacin, and very little sensitivity toward amoxicillin, nalidixic acid, and Co-trimoxazole [26].

M. morganii and *P. stuartii* isolates also exhibited high resistance to ticarcillin, gentamicin, and chloramphenicol. More than 61% of isolates were Multidrug resistant strains (resistant to at least 3 groups of antibiotics). 15% of the isolates were ESBL producers with no significant difference among various species.

In the present study, *Shigella* spp. isolates exhibited very high susceptibility toward Imipenem followed by Gentamicin, Ceftazidime, Cefoperazone sulbactam, Piperacillin tazobactam, and Ciprofloxacin and very less susceptibility toward Cefepime and Co-trimoxazole.

While comparing the resistance patterns between two studies by Mamtha *et al*. in 2007 and 2012 in the same region, all Shigella isolates were found to be resistant to nalidixic acid and a marked increase in resistance was observed. Resistance to ciprofloxacin increased from 30% to 87%, norfloxacin from 20% to 83%, ampicillin from 63% to 100%, tetracycline from 74% to 84%, and Co-trimoxazole from 79% to 90%. However, there was a significant decrease in resistance against gentamicin and amikacin from 71% to 40% and 45% to 5%, respectively [32,47].

In both the studies, Shigella isolates exhibited high sensitivity toward 3GC which is in accordance with our study. Similar findings were reported by Srinivasa *et al*. 2009 [31].

Shigellae may be susceptible to the aminoglycosides *in vitro*, but not *in vivo* due to poor penetration of the intestinal mucosa when given orally [48].

In addition to some fluoroquinolones, pivmecillinam (amdinocillin pivoxil) and ceftriaxone are the only antimicrobials found to be effective in the treatment of MDR strains of Shigella in all age groups. However, Azithromycin can be considered as an alternative drug among adults. However, these antibiotics should be used only when local strains are resistant to Ciprofloxacin [48].

Pazhani *et al*., in a study on childhood diarrhea (2001–2004), found that 50% of Shigella isolates were resistant to ampicillin and 96% to co-trimoxazole in 2001, which was reduced to 32% and 83%, in 2002. While 83% of isolates were resistant to tetracycline and 56% to nalidixic acid in 2001 which increased to 89% and 62%, respectively, in 2002. In addition, fluoroquinolone resistance emerged among *Shigella dysenteriae* and *Shigella flexneri* isolates in 2002 increased gradually during the study period from 11% to 25% [29].

We have isolated only three *S. typhi* isolates in our study which is not sufficient to give a clear generalizable picture of the local antimicrobial resistance pattern in the organism. However, all the isolates of *S. typhi* exhibited susceptibility toward Imipenem, Piperacillin tazobactam, Ceftazidime, Ceftriaxone, Cefoperazone sulbactam, and Chloramphenicol. High levels of resistance were observed toward Gentamicin.

Similar findings were reported by Mehta *et al*. 2018 Choudhary *et al*. 2013, Kumar *et al*. 2013, Singhal *et al*. 2014, and Gurung *et al*. 2017 [49-53].

Mehta *et al.* 2018 and Gurung *et al*. 2017 reported MDR toward first-line drugs in more than 15 % of *S. typhi* strains which were also resistant to Nalidixic acid [49,53].

It is observed that many of the isolates resistant to Nalidixic acid (NARST) were found to exhibit *in vitro* susceptibility to fluoroquinolones. But as per CLSI guidelines, such strains (NARST) should be considered Fluoroquinolone resistant as Nalidixic acid is a surrogate marker to predict fluoroquinolone treatment failure.

The resistance against fluoroquinolones like Ciprofloxacin is following an increasing trend due to the selective pressure by its unrestricted injudicious usage in typhoid therapy. Nalidixic acid resistance among *Salmonella* spp. is rapidly increasing in India. However, the consistent use of Ciprofloxacin as the mainstay treatment of typhoid in NA resistant cases has led to a steady rise in MIC along with further mutations at the same locus which has resulted in the emergence of completely resistant strains.

Several studies in the recent past have shown a re-emergence of susceptibility of *S. typhi* toward the first-line antibiotics. This could be due to their inconsistent usage by clinicians over the last decade who are preferring newer antimicrobials over them resulting in the withdrawal of selection pressure.

In the present study, 24% of NLF-GNB test isolates were found to be Multidrug-resistant. In a hospital setting, Multidrug-resistant or pan-resistant strains may transmit from one patient to another through the hands of health-care workers or via environmental contamination. These notorious pathogens are the potential reservoirs of resistance genes that could be transferred to other bacterial strains. The high levels of β-lactamase production and multidrug resistance of the isolates is an emerging public health threat globally. Environmental surveillance, searching for asymptomatically colonized persons through screening of patients as well as health-care workers, and using molecular epidemiology should be the requisite strategy for investigating the clusters of infection with pan-resistant organisms. These wild strains have great potential to survive in the hospital environment so improved antibiotic stewardship and infection control measures will be needed to inhibit the emergence and spread of multidrug-resistant NFGNB in the health-care setting [27,43,54].

In the current scenario due to rapidly emerging multiple drug resistance, the polymixins (polymyxin B and colistin) are being used frequently as the last line therapeutic option. However, the clinicians should restrict the use of the reserve drugs in exceptional conditions only [19].

CONCLUSION

The present study highlighted the fact that non-fermenter Gram-negative bacilli like *P. aeruginosa* and *Acinetobacter* spp. along with the other non-lactose fermenter, GNBs like the Proteae family, *S. typhi*, and *Shigella* spp. had emerged as important pathogens causing serious infections in the community as well as hospital settings. Infections caused by these notorious pathogens are difficult to treat as they are rapidly acquiring resistance to the commonly used antibiotics. Ever-increasing problem of antibiotic resistance has been worsened by the slow pace of research regarding the development of newer antimicrobial molecules.

Antimicrobial surveillance is needed to implement appropriate timely interventions to restrict the spread of multidrug-resistant clones. Strict infection prevention and control practices, with judicious antibiotics prescription policy, may help in tackling the emerging threat of multiple drug-resistant bugs by obviating the selection pressure. Appropriate judicious selection and rotation/cycling of antibiotics guided by the knowledge of their susceptibility profiles is of utmost importance.

The implementation of an antibiotic policy at the hospital level for the control and restriction of injudicious antimicrobial use is imperative in managing nosocomial infections.

This is the need of the hour to develop therapeutic protocols guided by susceptibility profiles for tuning antibiotic therapy regimens

to minimize the dissemination of antibiotic-resistant pathogens. Furthermore, the isolation of infected or colonized patients is of paramount importance.

Global antimicrobial susceptibility surveillance systems should also focus on the prevalence of multidrug-resistant/pan-resistant organisms, rather than restricting to just resistance rates to individual antibiotics so that the global impact of this problem could be assessed.

AUTHOR'S CONTRIBUTIONS

All the authors have made substantial contributions to conception, design, acquisition of data, analysis, interpretation, drafting manuscript, and have given final approval of the version to be published.

CONFLICTS OF INTEREST

None declared (by all authors).

FUNDING

Nil.

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