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Scientific Research

Isolation and Identification of Multi-Drug Resistant Strains of Non-Lactose Fermenting Bacteria from Clinical Isolates

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Abstract

Purpose: We studied the drug resistance of different microbes from clinical isolates. The morphological characteristics of bacteria were observed through culture characteristics and by carrying out gram staining techniques while the biochemical characteristics of bacteria were carried out by biochemical test. Methods: A total of 324 samples were collected from suspected patients visiting different hospitals at district Peshawar. For morphological identification, samples of clinical isolates were analyzed by blood agar, MacConkey agar and Eosine Methylene Blue, identified by gram staining and characterized by different biochemical tests. Antibiotic Sensitivity test by Modified Kirby-Bauer Disc diffusion method was used to test the *in-vitro* susceptibility of the identified isolates to different antibiotics such as *Ceftazidime*, *Ceftazidime*, *Ceftriaxone*, *Cefepime* and *Imipenem*. Results: These resistant non-lactose fermenting gram negative bacteria were isolated from samples of pus/wound (33.30%, n = 108/324), blood (33.30%, n = 108/324), urine (23.30%, n = 75/324) and from ascetic/pleural fluids (10.20%, n = 33/324). The study revealed that the percentage of non-fermenting bacterial infection was higher in females (53%) as compared to males (47%) along with higher infection observed in the age group of 11 - 30 years. *Pseudomonas aeroginosa* showed high resistance against *Cefepime* (88.80%), followed by *Cefoperazone* (55.50%), *Ceftazidime* (48.10%), *Ceftriaxone* (33.30%). *Imipenem* was active with low resistance (7.40%). More resistance was seen in *Morganella morganii* against *Imipenem* (66.70%) followed by *Cefope-*

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***razone* (55.50%), *Ceftriaxone* (55.50%). *Cefepime* showed low resistance (11%). Multi-drug resistant *Proteus mirabillis* was highly resistance to *Ceftriaxone* (74.07%), followed by *Cefepime* (59.20%), *Cefoperazone* (44.40%) and low resistance for *Imipenem* (25.90%). *Salmonella typhi* demonstrated high resistance against *Imipenem* (74.07%), followed by *Ceftriaxone* (40.70%), *Ceftazidime* (37.03%). *Cefepime* showed low resistance (3.70%), hence it is more active against *S. typhi.* Conclusions: The different species of non-lactose fermenting gram negative bacteria have shown a different resistivity pattern in the present study. Therefore identification of non-lactose fermenting gram negative bacteria and looking after their resistivity/susceptibility pattern are important for suitable management of the infections caused by them.**

Keywords

Multidrug Resistant, Non-Lactose Fermenting Gram Negative Bacteria, Disc Diffusion Technique

1. Introduction

Developing resistance to antibiotics is natural to microbes, which cannot primarily be ceased of as constantly evolving nature of microbes to chemicals around them. The phenomenon is very important regarding its practical and economic implications. It is because of this resistance people cannot be affectively treated and remain ill for a longer period of time. The development of tolerance of microbes to more than one drug is multi drug resistance (MDR) [1]. In the last few decades, antibiotic resistance is becoming a major problem across the globe [2].

Factors leading to antibiotic resistance include a widespread and aggressive use of broad-spectrum antibiotics, wrong investigated and diagnosis, using drugs without proper prescription by physicians and doctors, further including misuse of drugs by patients. With the resistant strains of bacteria the treatments of common infections become difficult or impossible. Non-fermenters are gram-negative bacteria that cannot ferment sugars to produced energy for cell physiology. Gram negative non-fermenting bacteria (NFGNB) were isolated from different clinical samples. Because of extreme multidrug resistance problems, species of this group offer a serious challenge for healthcare management [3]. As mostly, non-fermenting (gram-negative) bacteria are niche pathogens that cause infections in critically ill or immune-compromised patients. As they are primarily healthcare associated pathogens, they rarely cause infection in healthy individuals [4].

There are different mechanisms for resistance in non-fermenting gram-negative bacteria, including (i) production of enzymes (ii) enzymatic inactivation of antimicrobial agents (iii) specific targeted enzyme that is inhibited by antimicrobial agents (iv) alterations in target sites, (v) production of efflux pumps (vi) loss of outer membrane proteins or porins (vii) reduced uptake of the antimicrobial agent. That is because of these different resistance mechanisms that the therapeutic options are severely limited to treat infections caused by them [3] [4]. Non-fermenters include many species belonging to several genera. Previous studies suggest four species rarely found in hospitals, significant problems in hospital practice, including *Pseudomonas aeroginosa*, *Morganella morganii*, *Proteus mirabilis* and *Salmonella typhi* [5]. The emergence of multidrug resistance *P. aeruginosa* has emerged as a severe health problem [6], which is of low permeability of the cell wall, mutation in chromosomal genes regulating resistance genes and also acquiring resistance genes from other organisms via plasmids, transposons and bacteriophages [7] [8]. As the infection caused by multiple antibiotics resistant *P. aeroginosa* may result in worse clinical outcomes, this bacterium has gained particular importance [9]. Multi-antibiotic resistant of *M. morganii* strain is due to change in outer membrane permeability and mutation of the major porin or by a change in the number of porins in the outer membrane [10]. Increasing resistance to β-Lactam antibiotics in *P. mirabillis*, is mediated by the production of acquired β-lactamases. Plasmid-mediated ESBLs, including TEM-type derivatives active against expanded spectrum cephalosporin are also spreading in *P. mirabilis* [11].

In Pakistan the first case of multidrug resistance of *S. typhi* was reported in 1987 [12]. Since 1991, the cases of infections caused by *Salmonella* were increasingly resistant to extended-spectrum *cephalosporins* and *fluoroquinolones* [13]. *Salmonella typhi* resistance to *Fluoroquinolone* is associated with point mutations occuring mostly within a domain of gyrA. *Cephalosporins* resistance is usually mediated by extended spectrum β-lactamases derived from TEM- and SHV-type enzymes [14]. The present study aimed to assess the multi-drug resis-

tance of non-lactose fermenting bacteria in a low economic country like Pakistan, where people are not well aware of health issues. Our investigation will be a real contribution to the society and human health for further proper medication and human ease.

2. Materials and Methods

2.1. Sample Collection

A total 350 clinical samples were collected from suspected patients in different hospitals of Peshawar, KP Pakistan. *Samples were collected from urine, pus/wound, blood, ascetic/plural fluids* and were analyzed for colonial morphology *and routine biochemical identification.* The isolation of clinical samples was carried out according to standard protocol [15]. The collected samples from urine, pus/wound, blood and ascetic/plural fluids were spread on blood, MacConkey and Eosine Methyline Blue (EMB) agar plates and incubated at 37˚C for 24 - 48 hours. Gram staining was carried out as early described [16] to identify the NFGNB bacteria.

2.2. Biochemical Characterizations

Biochemical characterizations were performed through biochemical tests of clinical isolates. The protocol for clinical sample's identification was according to Cheesbrough *et al.* [15]. Indole, Methyl red, Citrate utilization, Triple sugar iron, Oxidase, Urease and Nitrate tests were also carried out.

2.3. Antibiotic Sensitivity Test

The Kirby-Bauer Disc Diffusion Method was used to test the *in vitro* susceptibility of the identified isolates to Ceftazidime (30 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), *Cefepime* (30 µg), Imipenem (10 µg). *Pseudomonas aeroginosa* colonies were picked up from the culture plate with the help of a sterile platinum wire loop and emulsified in 4 ml of sterile peptone water to match with 0.5 McFarland turbidity standards (1.5×10^8 cfu/ml). The surface of Mueller Hinton Agar (Oxoid, Basingstoke, UK) in a Petri dish was inoculated evenly through a sterile swab and for 10 minutes was allowed the agar to dry. A multichannel disc dispenser (Oxoid, Basingstoke, UK) was used to deposit the antibiotics discs onto the surface of the inoculated medium. The plate was then incubated at 37˚C for 24 hours. With measuring scale the diameters of zone of inhibition were measured in millimeters after 24 hours of period of incubation [17] [18]. The above procedure was repeated thrice for each *P. mirabillis*, *M. morganii* and for *S. typhi* isolates.

3. Results

A total of 324 multiple-drug resistant gram negative non-fermenters were isolated from 350 clinical samples processed at Microbiology Laboratory of Services Hospital Lahore. These bacterial strains were then identified on the basis of their cultural, morphological and biochemical characteristics (**Table 1**).

The occurrence of isolates was higher in females (53%, n = 171 in 324) as compared to males (47%, n = 315 in 324), while highest frequency of MDR-NFGNB was observed among young patients of age 11 - 30 (n = 171). The distribution of isolates among different age group showed that overall infection rate was higher in young individuals (11 - 30 years) but *P. aeroginosa* infection was higher in old age (61 - 74) (**Table 1**).

The Distribution of MDR-NFGNB *P. aeroginosa*, *M. morganii*, *P. mirabillis* and *S. typhi* in different clinical specimens (urine, pus/wound, blood, ascetic/pleural fluids) are shown in (**Table 2**). The results were also presented on simple cylindrical bar graph in **Figure 1**.

It was observed that *Pseudomonas aeriginosa* was found to be 88.80% resistant against *Cefepime*. The resistance found in MDR—*P. aeroginosa* against other antibiotics included Cefoperazone (55.50%); Ceftazidime (48.10%); Ceftriaxone (33.30%); Imipenem (7.40%). Imipenem is most active against *P. aeroginosa* (**Table 3**).

MDR-Patteren in *M. morganii* has observed 66.60% resistance against Imipenem. The resistance found against other antibiotics included Cefoperazone (55.50%); Ceftriaxone (55.50%); ceftazidime (48%); *Cefepime* (11%). *M. morganii* show lowest resistance to *Cefepime*, thus it is active against *M. morganii* (**Table 3**).

MDR-Patteren in *P. mirabilis*, a higher degree of resistance to Ceftriaxone (74.07%) was detected in *P. mirabillis* isolates. The frequency of resistance against was *Cefepime* (59.20%); Cefoperazone (44.4%); ceftazidime (37.03%); Imipenem (25.90%); (**Table 3**). As Imipenem showed lower resistance to *P. mirabilis.*

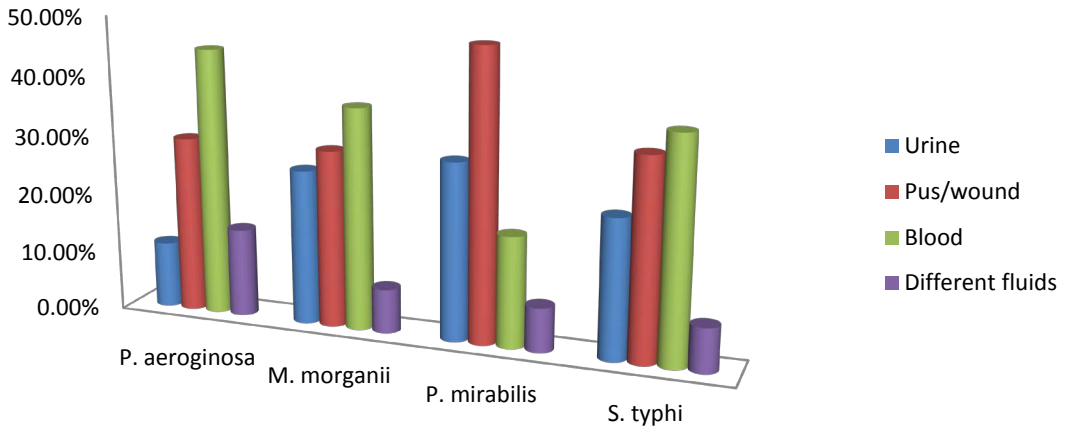

Figure 1. MRD-Bacteria percent isolated samples from different parts of the body of Patients.

Table 1. Distribution of MDR-bacteria in relation to gender and age wise in present study.

Variables	n	%
Gender:		
Females	153	53.00
Males	171	47.00
Total	324	100.00
Age:		
(11 - 30) years	N = 177	% = 100.00
P. aeroginosa	6	3.39
M. morganii	6	33.89
P. mirabilis	60	33.89
S. typhi	51	28.81
(31 - 60) years	N = 75	% = 100.00
P. aeroginosa	12	16.00
M. morganii	15	20.00
P. mirabilis	18	24.00
S. typhi	30	40.00
(61 - 74) years	N = 72	% = 100.00
P. aeroginosa	63	87.50
M. morganii	06	8.33
P. mirabilis	03	4.71
S. typhi	*	*

*n for numbers: % for percentage.

MDR-Patteren in *S. typhi Salmonella typhi* isolates exhibited high resistance against Imipenem (74.07%). Resistance to other antibiotics included Ceftriaxone (40.70%); Ceftazidime (37.03%); Cefoperazone (25.90%); *Cefepime* (3.70%) and thus a lower resistance to *Cefepime* was observed (**Table 3**). For the sake of convenience, results in **Table 3** were presented on simple horizontal bar graph (**Figure 2**).

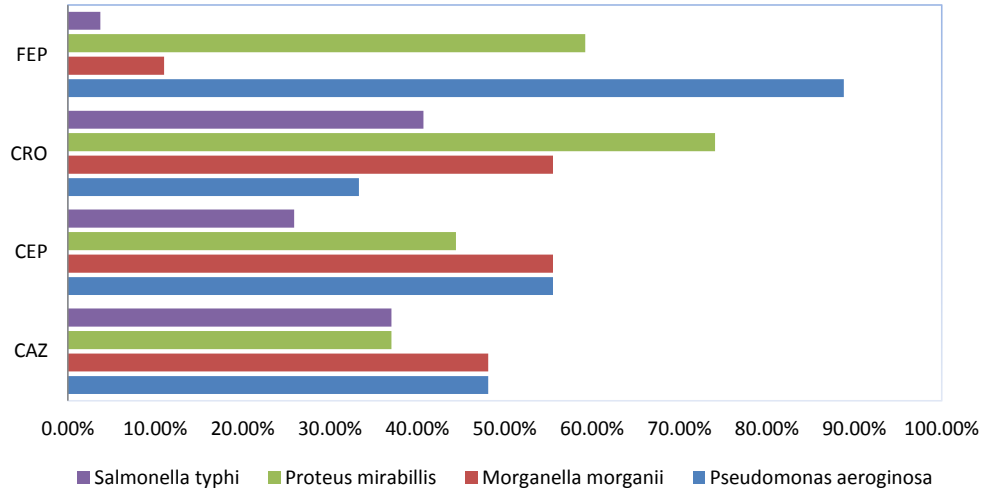

Figure 2. Antibiotics percent resistance in multi drug resistant bacteria from isolated samples.

Table 2. Clinical isolates of gram negative non-fermenters bacteria from different sites in hospitals.

Site	Organism	N = 324	%
Urine:			
	P. aeroginos	09	11.10
	M. morganii	21	25.90
	P. mirabilis	21	25.90
	S. typhi	24	29.60
Total:		n = 75	23.20
Blood:			
	P. aeroginos	36	44.40
	M. morganii	30	37.03
	P. mirabilis	15	18.50
	S. typhi	30	37.03
Total:		n = 108	33.30
Different fluids:			
	P. aeroginos	12	14.80
	M. morganii	06	7.40
	P. mirabilis	06	7.40
	S. typhi	06	7.40
Total:		n = 33	10.18
Puss/Wound:			
	P. aeroginos	24	29.60
	M. morganii	24	29.60
	P. mirabilis	39	48.20
	S. typhi	21	25.90
Total:		n = 108	33.30

*n for numbers: % for percentage.

Table 3. Antibiotic resistance percentage of gram negative non fermented bacteria (n = 81) isolated from different clinical samples.

Antibiotic	Code	Antibiotic % resistance in total, n = 81 for each species antibiotic resistance Profile isolated from different clinical specimens			
		Pseudomonas aeriginosa	*Morganella morganii*	*Proteus mirabillis*	*Salmonella typhi*
Ceftazidime	CAZ	48.10	48.10	37.03	37.03
Cefoperazone	CEP	55.50	55.500	44.40	25.90
Ceftriaxone	CRO	33.30	55.50	74.07	40.70
Cefepime	FEP	88.80	11.00	59.20	3.70
Imipenem	IPM	7.4	66.60	25.90	74.07

4. Discussion

Multi-drug resistance in bacteria is of very much concern to clinicians. Not only these organisms are very resistant but they are also rapidly spreading [19]. In clinical practices the antibiotic resistant pathogens causes financial burden, treatment failure and can spread to other patients [20]. In the last few decades, due to the widespread use of antibiotics, non-fermentative gram negative bacilli have emerged important health care-associated pathogens. Recent studies conducted on important areas like identification of non-fermentative gram negative bacilli, and monitoring their susceptibility patterns, which is important for the appropriate management of the infections caused by them, and to make clear the fact that it is important to establish the clinical relevance of the isolated non fermentative gram negative bacilli, before they are considered as pathogens. This would prevent unnecessary usage of antibiotics and the rise of drug-resistant strains [21]. There is limited data on the prevalence and resistance pattern of NFGNB. The current study was intended to address these issues.

In this study MDR-NFGNB was isolated from patients of different age groups (11 - 74). The highest number of MDR-NFGNB was isolated from age group of 11 - 30 years, followed by age group of 31 - 60 and 61 - 74 years. People were prone to infection at the age of 11 - 30 years because at this age the people are more active, have more social contacts, more journey from one place to another so have more chances of getting infection. Chances of getting infection do not depend upon time but depend on number of exposure to the injurious bacteria, viruses and toxins. At old age the people are restricted from social contact so have less chances of developing infection.

In the present study, Gender wise distribution of MDR-NFGNB was isolated both from males and females and highest numbers are from females. This could be due to the social activity of females in their life in developing countries like Pakistan. Where females are much ignored as compared to male so their food cleanliness is not good as males, as a result their immune system is weak. Furthermore, females give birth child's so admitted frequently to hospitals and thus has more chances of infection.

In present study *P. aeroginosa*, *M. morganii*, *P. mirabillis* and *S. typhi* were isolated and identified from blood, pus/wound, urine and ascetic fluids. These findings were in line with the results reported by other investigators [6] [22] and Javeed *et al*., [2] for *P. aeroginosa*, Singla *et al.* [23], Falagas *et al.* [24] and Lee *et al.* [25], for *M. morganii*. The present investigations showed higher frequency of *P. mirabillis* in pus (48.2%). Different researchers targeted *S. typhi* in blood, stool and bone marrow [26] [27]. The present study is unique in the sense as it identified *S. typhi* from urine (29.60%), blood (37.03%), pus/wound (25.90%), ascetic and pleural fluids (7.40%).

In-vitro MDR-Patteren in *P. aeroginosa* in present study to Imipenem was 7.40% which is consistence with the results of Romao *et al*. [28] who found 11% resistance against *Imipenem*, Tripathi *et al*. [29] found 10.80% resistance and Manian *et al*. [30] found 8% resistance against Imipenem in *P. aeroginosa. Pseudomonas aeriginosa* showed 88.80% resistance against *Cefepime* in the present study which is not consistent with the results of Zehra *et al*. [31] Romao *et al*. [28] and Tripathi *et al*. [29] who all detected 77.70%, 66%, 71%, 36.27% resistance against *Cefepime* respectively. The reason in the difference could be because of geographical differences. The fact is antibiotic resistance vary in different regions, environmental conditions and time to time.

Morganella morganii in-vitro MDR-Patteren showed 11% resistance against *Cefepime*. The finding of the current study is in agreement with the results of Falagas *et al*. [24], who found 8% resistance against *Cefepime*. Similarly Xiao-Bo *et al*. [32] found 14.30% resistance to *Cefepime*. In this study, the resistance of MDR-*M. morganii* against Imipenem 66.60% followed by Cefoperazone and Ceftriaxone 55.50% equals while Ceftazidime have 48.10%. These results were quite different from the results of Falagas *et al*. [24] who showed resistance 80% against Ceftriaxone and 90% against Ceftazidime. Xiao *et al*. [32] found 16.50% resistance against Imipenem and 5.50% against Amikacin. Similarly, Lee *et al*. [25] found 19.40% resistance against Imipenem. The difference is due to geographical locations, specimens types and extraction protocols.

In this study *in-vitro* MDR-*P. mirabillis* showed 74.07% resistance against Ceftriaxone followed by 59.20% against *Cefepime*, 44.40% against *Ceftazidime*, 37.30% against Ceftazidime and 25.90% against *Imipenem*. These results are in contradiction with the some of the earlier studies conducted by Falagas *et al*. [24] observed 100% resistance against *Cefepime* , Ceftazidime and 14.30% against Imipenem in *P. mirabillis.* Sharma *et al*. [33] examined 9.60% resistance against Cefoperazone and 4.80% resistance against *Cefepime* in *P. mirabillis.* Xiao *et al*. [32] detected 7.60% resistance against *Ceftriaxone*, 6.40% against *Ceftazidime*, 2.40% against *Cefepime*, 0.60% against Imipenem and 0.30% against Cefoperazone in *P. mirabillis.* Here also the difference in our results and others are because of sampling size and difference in specimen types.

5. Conclusion

The present study *in-vitro* MDR-Patteren *S. typhi* showed 40.70% resistance against *Ceftriaxone*, 37.03% *Ceftazidime*, 25.90% Cefoperazon, 74.07% Imipenem and 3.70% *Cefepime*. These results are quite in agreement with the findings of Mustaq [34], while different from the results of Nagshetty *et al*. [26] who showed 6.31% resistance against Ceftriaxone. Kumar *et al.* [35] found 12.10% resistance against Ceftriaxone. The difference may be due to the overuse of Ceftriaxone in our region. The study of multi-drug resistance is still an unsolved issue and needs further future endures [36] [37]. If this scenario is not properly tackled, there is a time coming, when there will be no more medication effect in terms of antibiotic use in order to cure and save human health and life.

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