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SHORT COMMUNICATION

Non-Fermenters in Human Infections with Special Reference to *Acinetobacter* Species in a Tertiary Care Hospital from North Karnataka, India

Prashant K. Parandekar[1] and Basvaraj V. Peerapur[1]*

[1] Department of Microbiology, BLDEA's Sri B. M. Patil Medical College, BLDE University, Solapur Road, Bijapur -586103, (Karnataka), India

Abstract:

Background: Non-fermenters are a group of aerobic non-spore forming gram negative bacilli that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative pathway. These are increasingly been reported from the cases of nosocomial infections. *Aims and Objectives*: This study was undertaken aiming to identify, characterize all non-fermenters and further study of *Acinetobacter* isolates. *Materials and Methods*: A total 116 non-fermenters isolated from various specimens obtained from the patients in tertiary care hospital. Gram negative bacilli which failed to produce acid on Triple Sugar Iron Agar (TSI) were identified by employing battery of tests. The *Acinetobacter* isolates were further speciated and antimicrobial susceptibility testing done by Kirby Bauer disc diffusion technique. *Results:* Non-fermenters isolated were *Pseudomonas aerugionsa* (69.8%), *Acinetobacter* species (18.9%), *Stenotrophomonas maltophilia* (4.3%), *Burkholderia cepacia* (3.4%), *Alcaligenes fecalis* (1.7%) and *Pseudomonas fluorescens* (1.7%). Most of the isolates showed susceptibility to imipenem (86.3%) whereas none of the isolates were sensitive to cephalexin and co-trimoxazole. *Conclusion*: This study highlights that, after *Pseudomonas aeruginosa,* Acinetobacter species is the most common non-fermenter. Majority of the isolates of Acinetobacter Species were of nosocomial origin and were multidrug resistant, which underlines the importance of proper vigilance of these infections in hospital setting.

Key Words: Non-fermenter, *A. baumannii.*

Introduction:

Non-fermenters are a group of aerobic non-spore forming gram negative bacilli that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative pathway [1]. Non-fermenters comprise about one fifth of all gram negative aerobic or facultative anaerobic bacilli recovered from extra intestinal specimens in clinical microbiology laboratory with *Pseudomonas aeruginosa, Acinetobacter* species *and Stenotrophomonas maltophilia* being most prevalent [2].

It is now recognized that *Acinetobacter* species plays a significant role in the colonization and infection of hospitalized patients. A variety of infections caused by *Acinetobacter* include pneumonia, endocarditis, meningitis, skin and wound infections, peritonitis and urinary tract infections. Moreover *Acinetobacter* species tends to be resistant to variety of antibiotics. There is increased trend towards

aminoglycoside resistance and multiple resistant strains have been reported including carbapenem resistance in nosocomial outbreaks [1].

Very few laboratories identify all non-fermenters routinely as the identification of non-fermenters requires battery of biochemical tests which are cumbersome to follow on routine diagnostic set up. Hence, the present study was undertaken to isolate, identify non-fermenters, to study *Acinetobacter* species isolates regarding speciation, its distribution in various clinical specimens and antibiotic susceptibility pattern.

Material and methods:

In the present study the non-fermenters isolated in the clinical specimens in bacteriology laboratory during January 2007 to December 2008 were included. The present study was approved by ethical clearance committee of BLDEA's Shri. B. M. Patil Medical College Bijapur. Informed consent of the patients was taken before collection of specimen. Apart from personal data clinical details of patients were reviewed for interpretation including the duration of hospitalization. The specimens yielding growth of non-fermenters were repeated within 48 hours for confirmation of the causative role. Only those isolates re- isolated on repeated culture are included in the study.

After direct smear examination, the specimens were inoculated on Blood agar and Mac - Conkeys agar. For specimens such as pleural fluid, sputum and cerebrospinal fluid - chocolate agar was also inoculated. The isolates were studied for morphology (Gram's stain), growth on MacConkey's medium, oxidase test, and Triple Sugar Iron Agar (TSI) reaction as preliminary tests [1, 2].

Gram negative bacilli which showed no change or alkali/no change on TSI after 18-24 hrs of incubation were considered as non-fermenters and subjected to battery of tests using various biochemical parameters combining principles of Gilardi's, Pickets schemes and bacterio logical texts [1-3].

The speciation of *Acinetobacter* species into two varieties i.e. *A. baumannii* (saccharolytic) and *A. lwoffi* (non-saccharolytic) was based on hemolytic activity and ability to oxidatively utilize glucose and xylose [1].

Antibiotic susceptibility testing of *Acinetobacter* species isolates was done by Kirby-Bauer disc diffusion technique [1] - Antibiotics tested were amoxyclav (10µg) , gentamicin (10 µg), amikacin (30µg), ciprofloxacin (5µg), cephalexin (30µg), gatifloxacin (5µg), ceftazidime (30µg), co-trimoxazole (1.25 µg/23.75 µg), tetracycline (10µg) and imipenem (10µg).

Results:

Table-1: Various non-fermenters isolated from the clinical specimens

S/N	Non-fermenters isolated (n=116)	No. of strains
1	*Pseudomonas aeruginosa*	81 (69.8%)
2	*Acinetobacter species*	22 (18.9 %)
3	*Stenotrophomonas maltophilia*	5 (4.3 %)
4	*Burkholderia cepacia*	4 (3. 4%)
5	*Alcaligenes fecalis*	2 (1.7%)
6	*Pseudomonas fluorescens*	2(1. 7%)

Table-2: Acinetobacter species isolated from various clinical specimens

Specimen	No. of isolates n=22	Lesion	No. of isolates
Pus	17 (77.2%)	Surgical site infection	3
		Burns	3
		Osteomyelitis	3
		Necrotic gangrene	2
		Cellulitis	2
		Pyopneumothorax	2
		Diabetic foot infection	2
Urine	3 (13.6%)	Urinary tract infection in catheterized patients	3
Pleural fluid	2 (9.0%)	Pneumonitis with pleural effusion	2
Total			**22**

The present study comprise of 116 non-fermenter isolated from various clinical specimens. After *Pseudomonas aerugionsa* (69.8%), *Acinetobacter* (18.9%) species was second commonest non-fermenter followed by *Stenotrophomonas maltophilia, Burkholderia cepacia, Alcaligenes fecalis* & *Pseudomonas fluorescens.*

Out of 22 isolates of *Acinetobacter* species, 18 (81.8%) were from the patients hospitalized for more than 48 hours. Further speciation of *Acinetobacter* species revealed majority of isolates as *A. baumannii* (19 isolates-86.36%). whereas 3 (13.63%) isolates were found to be *A. lwoffi*. Majority of *Acinetobacter* species isolates were from pus specimen followed by urine. The isolates from urine were observed from the cases of urinary tract infection in catheterized patients.

Antibiotic susceptibility testing showed imipenem as most effective antibiotic, as majority of the isolates of *Acinetobacter* (86.30%) were found to be susceptible to it. Antibiotic susceptibility to aminoglycoside like amikacin was only 27.2% and gentamicin (9 %) was still less. Moreover we observed that none

Table-3: Antibiotic susceptibility of *Acinetobacter* isolates

Antibiotic tested	No of isolates susceptible (n=22)	% of sensitivity
Imipenem	19	86.3
Gatifloxacin	7	31.8
Amikacin	6	27.2
Tetracycline	6	27.2
Ceftazidime	5	22.7
Ciprofloxacin	4	18.1
Amoxyclav	3	13.6
Gentamicin	2	9.0
Cephalexin	Nil	0
Co trimoxazole	Nil	0

of the isolates were susceptible to cephalexin and cotrimoxazole.

Discussion:

The present study comprises of 116 non-fermenters isolated from various clinical specimens. *Pseudomonas aerugionsa* has been most common isolate amongst non-fermenters (69.8%). *Acinetobacter* species (18.9%) has been the next common isolate followed by *Stenotrophomonas maltophilia* (4.3%), *Burkholderia cepacia* (3.4%), *Alcaligenes fecalis* (1.7%) and *Pseudomonas fluorescens* (1.7%). Other studies have been carried out on isolation and identification of non-fermenters in clinical specimens and have revealed varied isolation rates of non-fermenters. *Acinetobacter* species being the second commonest non-fermenter in all of them which is our finding also [4-5].

Although frequently considered as commensals or contaminants, the pathogenic potential of non-fermenters has been established beyond doubt by their frequent isolation from clinical materials and their association with diseases [1].

In the present study most of the isolates of *Acinetobacter* species have been from pus specimen (77.2%) followed by urine (13.6%). Our isolation rate of *Acinetobacter* species is higher as compared to Mishra B. who have reported 46.7% from pus and 7.6% from urine in his study [5].

According to the literature, amongst the *Acinetobacter* species, commonest species isolated in the human clinical specimens is *A. baumannii* [1]. We have also observed the same finding where 86.36% isolates were *A. baumannii* whereas remaining 13.63 % isolates were *A. lwoffi.*

In the present study, isolates of *Acinetobacter* species have been from the cases of cellulitis, burns, surgical site infection, urinary tract infection in catheterized patients etc., most of these patients have been hospitalized for more than 48 hours. Our finding emphasizes the fact that *Acinetobacter* spp. is implicated in variety of nosocomial infections.

Antibiotic susceptibility testing of *Acinetobacter* species has showed highest susceptibility to Imipenem (86.3%).whereas susceptibility to other antibiotics has been less like gatifloxacin (31.8%), Amikacin (27.2%), tetracycline (27.2%), ceftazidime (22.7%), ciprofloxacin (18.1%), amoxyclav (13.6%), gentamicin (9%). While none of the isolates have been susceptible to cephalexin and cotrimoxazole.

In the present study almost all isolates have been resistant to one or more antibiotics. Due to frequent resistance to aminoglycosides, fluoroquinolones, ureidopenicillins and third generation cephalosporin, carbapenems are important agents for managing *Acinetobacter* infections. However there has been alarming increase in reports of carbapenem resistant *Acinetobacter* species over the last decade [6]. Fortunately, our finding has been different, amongst 22 isolates, 19 (86.3%) were susceptible to imipenem, but this observation cannot be generalized as the numbers of isolates studied here are relatively less and there is need for further study including the detection of Metallo-beta-lactmase production in the *Acinetobacter* isolates.

To conclude, after *Pseudomonas aerugionsa*, *Acinetobacter* species is the most common non- fermenter associated with human infections. *Acinetobacter baumannii* is the commonest species isolated amongst Acinetobacter species. The present study highlights the fact that most isolates of *Acinetobacter* spp. have been of nosocomial origin and were multiple drug resistant. Except imipenem there has beens no other antibiotic which is effective for most of the isolates making therapy of infected individuals difficult. Thus there is need for proper vigilance of *Acinetobacter* infections in hospital settings not only for appropriate therapy but also for prevention of its dissemination in the hospital environment.

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**Corresponding Author: Dr. P. K. Parandekar, Professor, Department of Microbiology, BLDEA's Sri B. M. Patil Medical College, BLDE University, Solapur Road, Bijapur -586103, (Karnataka), India. Cell-09986740524, Email- prashantparandekar2000@yahoo.co.in*