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Prevalence of Extended Spectrum β- Lactamase Genes among the Oral Gram Negative Rods Isolated from HIV Infected Patients

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Abstract

Objective: We investigated the incidence of gram negative rods from the oral flora of HIV infected patients, possession of ESBL genes and their sensitivity pattern.

Methods: A total of 100 HIV infected patients were included in the study. Their oral swabs were cultured on blood agar and Mac-conkey agar. Organisms were identified based on standard technique. Antibiotic sensitivity was done by the Kirby Bauer disc diffusion method. Detection of ESBL gens (TEM, SHV, CTXM) were performed by multiplex PCR methods.

Result: A total of 57 gram negative rods were isolated from oral swab of 100 HIV positive patients. Out of 57 isolates Pseudomonas *spp* (21 isolates) followed by *Klebsiella* (17 isolates), *E.coli* (10 isolates) and *Acinetobacter* (9 isolates) respectively. Thirty four (60 %) isolates were ESBL producer by phenotypic methods and 35 isolates carried ESBL genes detected by PCR methods. By Kirby Bauer disk diffusion methods 14 (25%) isolates were resistance to three or more antibiotics were reflected as multidrug – resistance (MDR) organism.

Conclusion: All the HIV positive patients respiratory sample should be include in routine screening for the MDR isolation. This will help the physician to choses the appropriate antibiotic when needed and to prevent the morbidity and mortality of the HIV patients due to the MDR gram negative isolates.

Key word: *HIV, Oral flora, Gram negative bacilli. ESBL, MDR.*

Introduction

According to estimates by WHO and UNAIDS, 36.7 million people were living with HIV globally at the end of 2016. That same year, some 1.8 million people became newly infected, and 01 million died of HIV-related causes. [1] The infection is alarming due to the unique pathogenesis of the virus that mainly affect the CD4 cells count, due to this there is the emergence of opportunistic infections in the host, the infection become worse with low CD4+ T cell counts.[2] Among the various opportunistic infections, respiratory infections account for up to 70% of AIDS defining illness. Studies have found an incidence of 5-10% of the HIV-infected patients had colonization with different species of *Enterobacteriaceae.* [3]

It was well known fact that unselective use of antibiotics may cause a shift of upper respiratory tract microbiota and favour colonization with gram negative bacteria. [4] In recent year high level of drug resistance among the gram negative rods are a serious issue, such extended-spectrum-β–lactamase (ESBL) producers have widely spread, and at present, they lead to a serious threat to human.[5] Aim of the present study was to find out the incidence of ESBL genes in commensal isolates and to establish any upsurge in resistance against the commonly use antimicrobial agents and also to find out the incidence of gram negative rods as commensals in oropharyngeal isolates.

Material and Method

The study was conducted during the period from

June 2017 to August 2018 in a multispecialty tertiary care teaching hospital at Prayagraj UP India. A total of 100 HIV positive patients who satisfied the following inclusion/exclusion criteria, were included in the study.

Inclusion criteria:

Subjects who were positive with HIV and no respiratory complication were included in the study.

Exclusion criteria:

I. Subjects who received antibiotic during the past one month.

II. Upper respiratory tract infection.

III. HIV infected patients who were under antiretroviral drugs.

Method

After obtaining the institutional ethics committee's clearance, the patient related data were collected in a structured proforma. The data included the demographic details, CD4 count and complete blood count of the patients.

Specimen collection:

Normal saline mouth gargles or oral washings were collected in a sterile containers from the study population. [6]

Isolation and identification of the organism:

The samples were culture in 5% sheep Blood agar and MacConkey's Agar using standard procedures. Isolates were identified based on colony morphology on blood agar, MacConkey's agar, Two to three suspected colonies from each bacterial plate were picked, cultured and then identified by the various biochemical tests. Biochemical tests were performed to confirm different gram negative organism using gram stain, catalase test, indole, methyl red, Voges-proskauer test, nitrate reduction , urease production, simmon citrate agar, oxidase test and various sugar fermentation tests. [7] LF and NLF isolates were catagorized based on lactose fermentation on MacConkey's agar.

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing was done by Kirby–Bauer disk diffusion method as per Clinical and Laboratory Standards Institute guidelines [8] . The antibiotic disks (Hi-Media,India) used were ampicillin (10 µg), piperacillin (10 µg), piperacillin/tazobactam (100/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), amikacin (30 µg), gentamicin (10 µg), cotrimoxazole (1.25/23.75 µg), cefoperazone + sulbactam (75/30 µg), imipenem (IPM; 10 µg), meropenem (MRP; 10 µg), and ertapenem (ETP; 10 µg).

Screening for ESBL Production:

Gram negative rods which were resistance to third generation cephalosporines were tested for ESBL production by combination disk method using cefotaxime (30 µg), cefotaxime/ clavulanic acid (10 µg), ceftazidime (30 µg) and ceftazidime/ clavulanic acid (10 µg). A ≥5 mm increase in diameter of inhibition zone of cephalosporin + clavulanate disc when compared to cephalosporin disc alone was interpreted as evidence of ESBL production. [8]

Preparation of template DNA:

To 500 µl sterile distilled water taken in a micro centrifuge tube was added 2-3 identical colonies of the isolate. This suspension was heated in a water bath at 95 ^{o} C for 10 min and then centrifuged at 10,000 rpm for 10 min. the supernatant were collected which contain the bacterial DNA was used as template for polymerase chain reaction (PCR). [9]

Genotypic detection of ESBL encoding genes:

A multiplex PCR assay was performed to detect bla $_{\text{TEM}}$, bla $_{\text{SHV}}$ and bla $_{\text{CTX-M}}$ genes as per the primers and conditions previously described, with minor changes [10, 11] . To summarize, PCR was done in a final reaction volume of 50 µl containing 750 Mm Tris HCl, 200Mm $(NH4)_2SO4$, 2.5mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µl of each primer, 1.5U of Taq DNA polymerase And 5µl of template DNA. The program for amplification included a step of initial denaturation at 95 ^{0} C for 3 min followed by 30 cycles of 95 ^{o} C for 1 min, 58 ^{o} C for 1 min and 72 ^{o} C for 1 min and final extension step at 72 ^{o} C for 7 min. The PCR products were loaded in 2% (w/v) agarose gel prepared in tris borate EDTA buffer at 120 V for 1h and detected using ethidium bromide staining after electrophoresis.

Statistical Analysis

Statistical analysis was performed by using SPSS, version 17.0. Correlation of numerical data with drug resistance and virulence was done using Pearson's correlation coefficient and all categorical data were correlated by chi-square test. A value of $P < 0.05$ was considered statistically significant.

Results

A total of one hundred respiratory samples from HIV infected patient were included in the study. Out of 100 patients 78 were males and 22 were females. Of the total of 100 patients oral sample 57 sample contain gram negative rods. Of the total of 57 isolates maximum number of the isolates were *Pseudomonas spp* (21 isolates) followed by *Klebsiella spp* (17 isolates), *E. coli* (10 isolates) and *Acinetobacter* (9 isolates) respectively. The most common age group colonised by gram negative rods were 41-60 years followed by 17-40 years. (Table1). Maximum numbers (47.50%) of gram negative rods were isolated from the patients with the CD4+T cell count of 200-600 cells/µl (Table1). Prevalence of different gram negative rods with different CD4 cell count groups is summarized in Table 2.

Of the total of 57 gram negative isolates 34 (60 %) isolates were ESBL producer by phenotypic methods and 35 isolates harbouring ESBL genes which code for single or multiple enzymes**.** A total of 32 (91%) strains out of 35 isolates were positive for bla $_{\text{CTX-M}}$ gene, 6 (17%) isolates were positive for bla $_{\text{TEM}}$ and only 2 organism were positive for bla $_{\text{SHV}}$. Two organism were positive for both bla $_{\text{TEM}}$ and bla $_{\text{CTX-M}}$, However , non of the isolate was positive for all ESBL genes. When the isolates –wise distribution of ESBL genes was considered, 52% *Pseudomonas* isolates were harbouring bla $_{\text{CTXM}}$ genes (Table 3). The results obtained from the Kirby Bauer disk diffusion methodes indicated that, of the 57 gram negative bacilli all of them were fully susceptible to Ertapenem , Meropenem and Imipenem respectively. Around 25% isolates were resistance to three or more antibiotics and thus were considered as multidrug – resistance (MDR) strains. Around 95% of the all isolates were resistant to ampicillin and around 75% of the isolates were resistant to fluroquinolones group of drugs. In addition, the resistance profiles of the drugs among the isolates are summarized in figure 2.

Table 1: demographic details and CD4 cell count of the patients:

		HIV infected patients respiratory sample contain no GNB	**HIV infected patients respiratory sample contain GNB**	**Total**
Sex (N=100)	Male	40	38	78
	Female	3	19	22
Age (N=100)	0-16	3	4	7
	17-40	34	20	54
	41-60	9	26	35
	>60	1	3	4
CD4 Cell count (N=100)	<200	8	15	15
	200-600	11	27	29
	>600	24	15	15

Table 2: Prevalence of Gram negative rods with different CD4 cell count group:

Bacteria isolated n=57	CD4 <200	CD4 200-600	CD4 >600
Pseudomonas (21)	12.2%(7)	19.2%(11)	5.2 %(3)
Klebsiella (17)	5.2%(3)	52 % (9)	23.8% (5)
E.coli (10)	3.5%(2)	7%(4)	10.52%(6)
Acinetobacter(9)	5.26 % (3)	8.7% (5)	1.7% (1)
Total	15	29	15

Table 3: Phenotypic and Genotypic distribution of ESBL producing organism.

Organism		Phenotypic n=34	Genotypic n=35		
			TEM n=6	SHV n=2	CTXM n=32
1.	Pseudomonas n=21	13	1	1	11
2.	Klebsiella n=17	8	1	1	6
3.	E.coli n=10	6	2	0	7
4.	Acinetobacter n=9	7	2	0	7

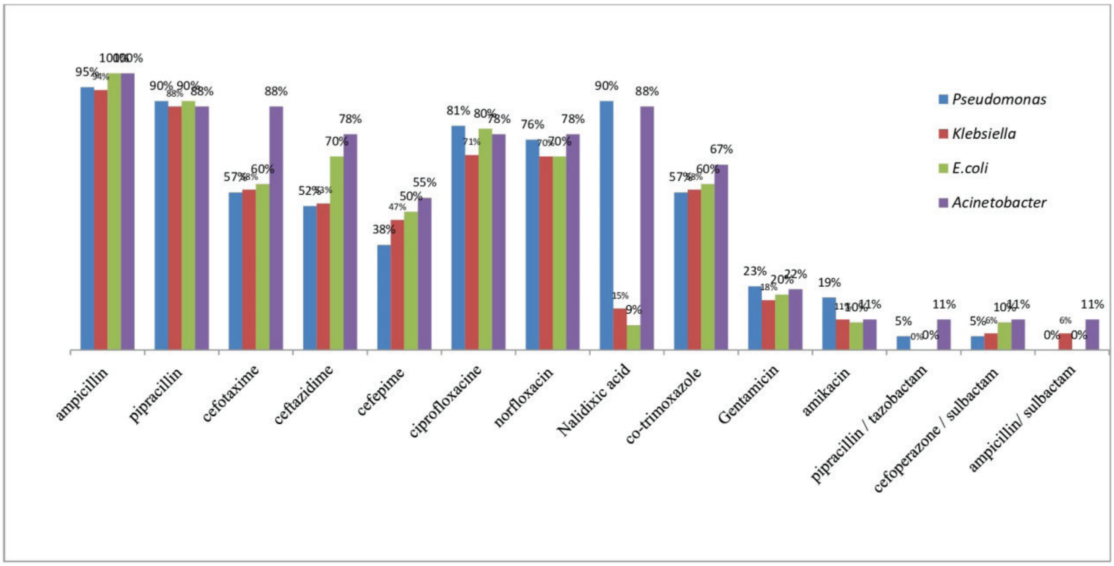

Figure 1: Antimicrobial resistance pattern of commonly used antibiotic among the isolates.

Discussion:

In the present study, we observed a high rate of colonization of gram negative rods (57%) among the HIV positive patients respiratory sample which is similar to the study done by Hegde et al. [6] where they have found around half of their isolates were gram negative rods.

We are not the first to observed that male were mostly effected by HIV in compare to female several other investigator also reported the same. We also witnessed that the incidence of colonization of gram negative bacilli were significantly higher in male patients. The age group mostly affected was the 41-60 years; this is consistent with the findings of *Hedge et al* [6] and Leynaert et al.[12] . Who reported the highest incidence of HIV in the late thirties.

In the study, we also observed that , the incidence of gram negative rods were significantly higher in patients who have lower CD4 cell count, which is similar to studies done by Sarkar K et al. [13] and Hegde et al. [6]

In our study population we found that around half of the HIV infected patients oral sample contain gram negative isolates, Among these isolates the most common were *Pseudomonas spp* (37%) followed by *Klebsiella spp* (30%). However a study done by Schmidt- *Westhausen et al*. [14] found that 5% of the HIV infected individuals oral cavity contain different species of *Enterobacteriaceae*. Yet in another study from India have reported 32% of their isolates were gram negative rods. [6]

On analysis of the antibiotic sensitivity testing by phenotypic methods of the isolates we observe a significantly high degree of resistance to first line of antibiotics which is in accordance to study done by Manfredi et al. [15] and Molyneux .[16] Many retrospective studies on commensal isolates from HIV positive patients reported a high degree of resistance among the commonly used antibiotics.

Regarding the possession of ESBL genes such as CTXM, TEM & SHV, our finding indicate that one in three HIV positive individual were harbouring gram negative rods with ESBL genes (100/35). However the incidence of ESBL producing isolates were significantly higher among the total gram negative rods isolated (57/35) from HIV positive individual. There are several study on *E.coli* phylogenetic groups indicates that commensal groups such as A & B1 were harbouring drug resistance genes and causing life threating infection. [17, 18]

In our study we also observed that CTXM is the most common ESBL gene harbouring by the isolates, the presence CTXM gene among the commensal isolates may act as a reservoir of antibiotic resistance genes.

It is well know that opportunistic infections are the most common causes of death in HIV infected patients and most of them are caused by commensal bacteria which are otherwise harmless in a normal individual. In our study we have reported that those commensal gram negative rods were harbouring ESBL genes, a finding that is of a major worry to attending clinicians and present day treatment modalities. Based on our finding we can suggest that the HIV positive individuals respiratory sample should be routinely scan for the pathogens and if there are any gram negative rods grown they should treated with the appropriate antibiotic. This will help in the fight against multidrug resistance organism and if corrective actions are not taken, in the absence of novel agents in the near future, the spread of MDR isolates and infection cause by those isolates in HIV infected individual may lead to therapeutic dead ends.

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Ethical Clearance: Institutional ethical committee Motilal Nehru medical college. ECR/922/INST/UP/2017/ DATED: 26/12/2018.

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Conflict of Interest : NIL.

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