

Frequency of Enteric Gram Negative Rods Isolated from Various Clinical Samples

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Abstract

Objective: To determine the frequency of gram negative rods in both nosocomial and community acquired infections in our setting.

Material and Methods: This descriptive study was conducted at the Microbiology Department of Fauji Foundation Hospital, Rawalpindi over a period of two years (April 2004 to March 2006). All pus, urine, blood and HVS received during the study period were included. Samples without properly filled forms, dried swabs and improperly transported samples were excluded.

Results: Two thousand and ninety one Enteric Gram Negative Rods (EGNR) were isolated over a period of two years. They were most commonly found in urine samples (40.7%), followed by pus (37.1%), HVS (12.0%), sputum (6.12%) and blood (3.92%) samples. The most common EGNR isolated was *Escherichia coli* (49.7%) followed by *Klebsiella pneumoniae* (23.4%), *Pseudomonas aeruginosa* (19.5%) and *Proteus* species (1.81%). The most commonly isolated Enteric Gram negative rod from urine samples (n 852) was *E. coli* (622) followed by *Klebsiella pneumoniae* (189), *Providentia* (18) and *Enterobacter* species (13). The most commonly isolated Gram negative rod from pus samples (n 852) was *Pseudomonas aeruginosa* (290) followed by *E. coli* (227), *Klebsiella pneumoniae* (18) and *Proteus* species. The most commonly isolated EGNR from HVS samples was *E. coli* (130) followed by *Klebsiella pneumoniae* (68) and *Pseudomonas aeruginosa* (51). The most common EGNR found in sputum (n 128) was *Klebsiella pneumoniae* (56) followed by *Pseudomonas aeruginosa* (40) and *E. coli* (30). In blood samples the most common organism isolated was *E. coli*, followed by *Pseudomonas* and *Klebsiella*.

Key Words: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*

Introduction

The human life has always been in danger from diseases caused by microorganisms. The history still mourns the death toll of epidemics of influenza, plague & malaria which occurred during the 19th century. Gram negative bacilli

(GNB) are a common cause of sepsis, pneumonia, urinary tract infections, and post surgical infections in patients in acute care hospitals.¹ During 1970s prevalence of nosocomial infections at specific sites has varied from survey to survey. Gram negative bacilli and *Staphylococcus aureus* were most frequently isolated from patients with hospital-acquired infection.² During 1980s there has been a major shift in the etiology of hospital-acquired infections leading to an increase in the laboratory isolation of Coagulase-Negative *Staphylococci*, *Candida*, *Staph aureus*, *Enterococci*, *Pseudomonas aeruginosa* and *Enterobacter* spp.³ Etiologic shifts in nosocomial infections and development of antimicrobial resistance among these pathogens, particularly those isolated from intensive care units are alarming.⁴ Taken as a whole, the shifts are away from more easily treated pathogens towards more resistant pathogens with fewer options left for therapy.⁵ Keeping in view the importance of EGNR (especially *E. coli*, *Klebsiella*, *Pseudomonas*) as major pathogens, and the emerging resistance against the commonly used antibiotics, the present study was designed to determine the frequency of gram negative rods in our setting.

Material and Methods

This study was carried out in the department of microbiology, Fauji Foundation Hospital Rawalpindi, over a period of two years (April 2004 to March 2006). The clinical samples including pus, high vaginal samples, urine, blood and sputum, received from various indoor and outdoor departments were inoculated on the special and enriched media depending on the type of the sample and were identified by the help of standard procedures. The aerobically incubated organisms were identified with the help of colonial morphology, gram staining and biochemical analysis like TSI, indole, urease, citrate and API depending upon the availability.⁶

Results

Two thousand, ninety one EGNR were isolated over a period of two years. The samples received during the study period were: urine samples 852 (40.7 %), followed by pus samples

Organisms	Urine	Pus	HVS	Sputum	Blood	Total	
	No-%	(No)	(No)	(No)	(No)	(No)	%
<i>Escherichia coli</i>	622-73.0	227-29.1	130-51.5	30--23.4%	30-36.5 %	1039	49.7
<i>Klebsiella pneumoniae</i>	189-22.0	171--2.31%	68-26.9%	56--43.7%	6--7.31%	490	23.4
<i>Pseudomonas aeruginosa</i>	-	290-37.3%	51-20.2%	40-- 31.2%	26-31.7 %	407	19.5
<i>Proteus species</i>	-	33-3.87	-	1	4	38	1.81
<i>Providencia species</i>	18--2.1	11	-	-	-	29	1.38
<i>Enterobacter species</i>	13--1.52	12	-	-	4	29	1.38
<i>Acinetobacter species</i>	-	12	-	-	1	13	0.62
<i>Citrobacter species</i>	6	2	1	-	-	9	0.43
<i>Salmonella species</i>		6	-	-	2	8	0.38
<i>Morganella species</i>		7	1	-	-	8	0.38
<i>Salmonella typhi</i>	-	-	-	-	6	6	0.28
<i>Hafnia species</i>	3		1	-	-	4	0.19
<i>Serratia species</i>	1	2	-	1	-	4	0.19
<i>Aeromonas species</i>	-	1	-	-	3	4	0.19
<i>Yersinia atypical</i>	-	1	-	-	-	1	0.05
<i>Fusobacteria species</i>	-	1	-	-	-	1	0.05
<i>Xanthomonas species</i>	-	1	-	-	-	1	0.05
TOTAL	852	777	252	128	82	2091	

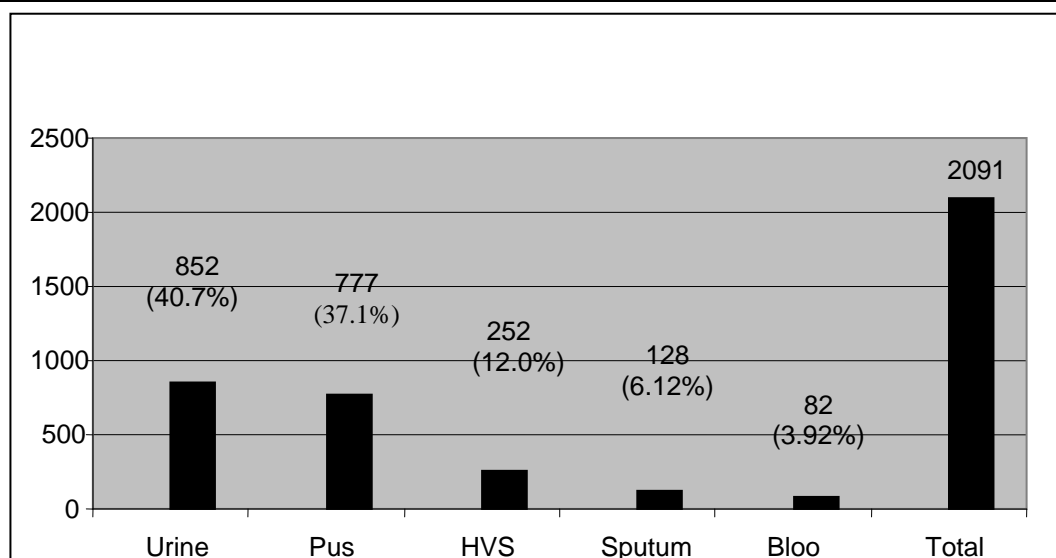


Fig 1; Frequency of EGNR in various samples

777 (37.1%), HVS 252 (12.0%), sputum 128 (6.12%) and blood 82 (3.92%) (Figure 1).

Most common EGNR isolated were *Escherichia coli* 1039 (49.7%) followed by *Klebsiella pneumoniae* 490 (23.4%), *Pseudomonas aeruginosa* 407 (19.5%), *Proteus* species 38 (1.81%). The rare occurring organisms were *Hafnia* species, *Serratia* species, *Aeromonas* species 04 (0.19%) each followed by *Yersinia*, *Fusobacteria*, *Xanthomonas* species 01 (0.05%) each. The most commonly isolated Enteric Gram negative rod from urine sample was *E. coli* 622 (73.0%) followed by *Klebsiella pneumoniae* 189 (22.0%), *Providentia* 18 (2.1%) and *Enterobacter* species 13 (1.52%). The most commonly isolated Gram negative rod from pus sample was *Pseudomonas aeruginosa* 290 (37.3%) followed by *E. coli* (227-29.1%), *Klebsiella pneumoniae* 171 (2.31%) and *Proteus* species (33-3.87%). Among HVS sample the most frequent EGNR was *E. coli* 130 (51.5%) followed by *Klebsiella pneumoniae* 68 (26.9%), *Pseudomonas aeruginosa* 51 (20.2%). The most frequently found EGNR found in sputum was *Klebsiella pneumoniae* 56 (43.7%) followed by *Pseudomonas aeruginosa* 40 (31.2%) and *E. coli* 30 (23.4%). In blood samples *E. coli* was the most commonly isolated pathogen 30 (36.5 %) followed by *Pseudomonas aeruginosa* 26 (31.7 %) and *Klebsiella* 6 (7.31%) (Table1).

Discussion

Two thousand, ninety one Enteric Gram Negative Rods (EGNR) were isolated over a period of two years. EGNR were most common pathogens isolated from urine samples (40.7 %), followed by pus samples (37.1%), HVS (12.0%), sputum (6.12%) and blood samples (3.92%). In a previous study conducted on different bacterial isolates, the frequency rates of causative bacteria in various types of infectious diseases were mentioned. Significant urine culture isolates were *E. coli* and other enterobacteriaceae in uncomplicated UTI, and *Pseudomonas* spp. in complicated UTI with a urinary catheter. In respiratory tract infections (RTIs), *P. aeruginosa*, were among common causative organisms. In common with hospital-acquired pneumonia, *P. aeruginosa*, and enterobacteriaceae were the frequent microorganisms isolated.⁷ These results correlate to our study which has shown enteric gram-negative and related rods to be the most common isolates from different clinical samples.

Most common EGNR isolated were *Escherichia coli* (49.7%) followed by *Klebsiella pneumoniae* (23.4%), *Pseudomonas aeruginosa* (19.5%), and *Proteus* species (1.81%). This is comparable to previous studies which have shown *E. coli* (61%) to be the most common pathogen followed by *K. pneumoniae* (22%), *P. aeruginosa* (4.0%), *A. baumannii* (3.0%) and *Citrobacter* spp (2.0%).⁸⁻¹⁰

The most commonly isolated Enteric Gram negative rod from urine sample is *E. coli* (73.0%) followed by *Klebsiella pneumoniae* (22.0%), *Providentia* (2.1%) and *enterobacter* species (1.52%). Of the 920 tested sample, 100 samples showed growth of pathogens among which the most

prevalent were *E. coli* (61%) followed by *Klebsiella* spp (22%).¹⁰ In a study conducted in Iran, isolated bacteria in urine were as follows : *Escherichia coli* 72.9%; *Klebsiella pneumoniae* 24.5%; *Citrobacter* spp 1%; *Proteus mirabilis* 0.6% and *Pseudomonas* spp 1%.¹¹

The second most common sample from which Gram-Negative Rods were obtained in this study were pus samples (37.1%) followed by High vaginal swabs (12%), sputum samples (6.12%) and blood samples (3.92%). The most commonly isolated Gram negative rod from pus sample is *Pseudomonas aeruginosa* (37.3%) followed by *E. coli* (29.1%), *Klebsiella pneumoniae* (2.31%) and *Proteus* species (3.87%). The results in previous studies showed *E. coli* (25-31%), *K pneumoniae* (9.5-10%) & *Pseudomonas* (8.6-38%).¹²⁻¹⁵ In another study frequencies of pathogens in primary surgical infections in an order of decreasing frequency were *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter*, *Klebsiella pneumoniae* and others.¹⁵

The most commonly isolated Gram-negative rod from HVS samples is *E. coli* (51.5%) followed by *Klebsiella pneumoniae* (26.9%) and *Pseudomonas aeruginosa* (20.2%), comparable to a previous study where *E. coli* was 21%.¹⁶ Chow et al and Izhar et al also made similar observations, as *E. coli* were the commonest organisms (25&45%), followed by *K pneumoniae* (18 & 25%), *P aeruginosa* (22 & 28.7%), *Acinetobacter* spp (7% each) and *Enterobacter* spp (7 & 11%).^{17,18} *Pseudomonas aeruginosa* (30.3%) was the most frequent isolate among Gram-negative organisms, followed by *E. coli* (18.6%), *K pneumoniae* (16.9%), *Acinetobacter baumannii* (8.8%) and *Enterobacter cloacae* (7.1%).¹⁹ Majority of the above mentioned organisms are normal flora of female genital tract and may not be implicated with disease production. It seems unlikely that the gynecological infections are caused by the same mechanism throughout the world. The members of normal flora may themselves produce disease under certain circumstances if introduced into foreign locations in large numbers and if predisposing factors are present.

The most common EGNR found in sputum was *Klebsiella pneumoniae* (43.7%) followed by *Pseudomonas aeruginosa* (31.2%) and *E. coli* (23.4%) comparable to a study by Jawad et al 2011 where most prevalent gram negative rods causing respiratory tract infections were *Klebsiella pneumoniae* (21.4%) & *P. aeruginosa* (15.3%).²⁰ Whereas the prevalent pathogens in other studies were *H influenzae* (73%), *Moraxella catarrhalis* (12%), and *H parainfluenzae* (5%), *Klebsiella* spp (7.7%), and *P. aeruginosa* (2.9%).^{21,22}

Blood stream infections (BSI) revealed *E. coli* (36.5 %) and *Pseudomonas aeruginosa* (31.7 %) followed by *Klebsiella pneumoniae* (7.31%). The results were comparable to other studies whereas *E. coli* (17.2-37%), *Klebsiella* spp (6.3-9.6%), and *Pseudomonas aeruginosa* (6.5%) were the predominant organisms.²³ During 1997-2002, a total of 81,213 BSI pathogens from North America, Latin America, and Europe were tested for antimicrobial susceptibility. *E. coli*, were the most common BSI pathogens in all three regions each year.⁶ In other studies *E. coli* (8.1-21%), *Pseudomonas* spp (6.7-17%), *Acinetobacter* spp (5-10%),

Salmonella spp (3.8%) and miscellaneous group (9.2%) were the most common pathogens.^{24,25}

In a study by Mamishi et al, (2005) Klebsiella spp (31%) were most predominant, followed by Escherichia coli (21%) and Pseudomonas aeruginosa (17%). Decousser et al, (2003) established Escherichia coli, as one of the major bacterial isolates in BSI.^{26,27} In a study by Mehta et al, (2005), among the 567 qualifying samples, Pseudomonas aeruginosa (19.75%), Escherichia coli (15.17%), Klebsiella pneumoniae (14.99%), and Salmonella enterica serovar typhi (12.87%) were the most frequently isolated Gram-negative bacteria.²⁸

Conclusion

The sample showing gram-negative rods as a major pathogens were urine samples followed by pus samples. Escherichia coli is the most common gram-negative rod isolated followed by Klebsiella pneumoniae and Pseudomonas aeruginosa.

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