ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bacteriological Profile of Septicaemia and Antimicrobial Susceptibility of Isolates from Tertiary Care Hospital in India

Savita Jadhav*, Nageswari Gandham, Retina Paul, RN Misra, MT Ujagare, Kalpana Angadi, and Chanda Vyawahare

Department of Microbiology, Pad. Dr. D. Y. Patil Medical College and Hospital Pimpri-Pune-18

ABSTRACT

Septicaemia is a potentially life-threatening infection in which large number of bacteria are present in the blood. Septicaemia usually arises as a result of localized infection in the body. The primary site of infection may occur in the respiratory system, the skin, the gastrointestinal system or the genitourinary system. It may correspond with very debilitating infections such as meningitis. Bacteria usually spill over from the primary infection site into the blood and are carried throughout the body thereby causing infection in various systems of the body. It can be complicated by circulatory collapse, myocardial depression, increased metabolic rate and vasoregulatory perfusion abnormalities. Thus it must not be viewed as simply being an infection alone. A retrospective review was conducted during Jan. 2008 to Dec. 2011 at the Pad. Dr. D. Y. Patil Medical College and Hospital Pimpri Pune-18, India. 3180 blood samples were received from various wards, ICUs and OPDs. All blood samples were processed and identified by standard conventional methods. Antimicrobial susceptibility testing was done according to CLSI guidelines. A total of 1657(52.10%) were culture positive amongst which 894 (53.95%) were Gram Negative Bacteria (GNB) while 736 (44.41%) were Gram Positive Cocci(GPC) and 27 (1.62%) were *Candida spp.* Among all Gram negative bacteria, 28.97% were ESBL producers and 5.48% were MBL producers. 700(42.24%) isolates were from ICUs patients. Conclusion- Our finding provides information on spectrum of microorganisms and antibiotic resistance in septicaemic patients. Mortality and morbidity of neonatal sepsis is elevated and is considerably contributed by positive blood culture with preponderance of MDR GNB.

Key words: Septicaemia, Extended spectrum β- lactamases, Multidrug resistance Gram negative bacilli, gram positive cocci, Coagulase negative staphylococci.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Septicaemia constitutes one of the most serious situations in infectious diseases. Septicemia is a medical disaster, and rapid succession keeps health personnel on their toes in a bid to confine the situation. Neonatal septicaemia is a major cause of neonatal mortality which is elevated by occurrence of multidrug resistance (MDR) by gram negative bacteria (GNB) [1-3]. Current WHO recommendations (2005) for management of neonatal sepsis (< 2 month old) include hospitalization and parenteral antibiotic therapy at least 10 days with penicillin agent (e.g. benzyl penicillin or ampicillin) combined with an aminoglycosides e.g. Gentamicin. Extensive use of cephalosporins leads to increase in infections caused by extended – spectrum β- lactamases-producing *Escherichia coli and Klebsiella species (ESBL-EK)* [4-8]. ESBL-EK has become upward concern in hospitalized as well as community settings worldwide. Occurrence of primary health-care-associated blood-stream infections (PHCA-BSIs) has been growing progressively and most are related to the use of central venous catheters (CVCs). These infections have tremendous effect on patients outcome and hospital cost, resulting in hospital stay [9,10,11]. In India, MDR-GNB are leading cause of PHCA-BSIs. Determination of microbial etiology of PHCA-BSIs has vital significance to formulate empirical antimicrobial policy, patient's outcome, other patients care as well as infection control practices [12]. Fungemia is usually serious condition, occurring primary in immunosuppressed patients and in those with serious or mortal illness. *Candida albicans* was the most common species by vast margin still last decade but *Candida tropicalis* and *Candida glaberata* are now commonest fungemia producing fungus in this decade [13-15].

Evidently, early reorganization of sepsis and appropriate therapy before shock ensures is paramount in reducing high mortality. The expeditious detection and identification of blood-borne pathogens is one of the most important functions of the microbiology laboratory. Positive blood culture may help to provide a clinical diagnosis. The kind of microorganisms isolated from blood cultures vary according to the patient's population and nature of the disease. In comparison to developed countries, inadequate data is available in India regarding spectrum and pattern of causative agents of septicaemia [12-16]. In the view of serious threat of septicaemia, it is necessary that the clinicians should have pattern and nature of the bacteria commonly implicated in septicaemia. This would guarantee prompt and more efficient management of the affected patients. This study was to determine the pattern of bacteremia and septicaemia in order to generate local data that would be useful for local policy formulation in managing septicemia cases especially in neonatal units and intensive care units (ICU).

MATERIALS AND METHODS

Study site

The study was carried out at Pad. Dr. D. Y. Patil Medical College and Hospital Pimpri Pune -18. Maharashtra, India.

Study period-The study was conducted between January 2008 to December 2011.

ISSN: 0975-8585

Study procedure

The study was laboratory and hospital based retrospective in nature and data was generated from microbiology departmental record based on patient's history and antibiotic susceptibility pattern of respective isolated causative agent from blood samples received from various wards, ICUs and OPDs from Pad. Dr. D. Y. Patil Hospital which is 1800 bedded tertiary care hospital in semi urban locality of Pune district.

Microbiological procedures and methods-

True bacteremia, hospital-acquired bacteremia and blood culture contaminant were defined by using conventional criteria [17-19]. Specimens were collected, transported by standard conventional methods [20]. Isolation and identification of microorganisms at the species level were done by standard conventional methods [17].

Antibiotic susceptibility tests

The Kirby- Bauer method recommended by the CLSI guidelines (2007) was used for antimicrobial susceptibility testing [14].

Detection of Extended Spectrum β-Lactamases-Screening Test (CLSI, 2007)

Initially screening test for ESBL production was done as part of routine susceptibility testing. Two antibiotic discs, ceftazidime (30 µg) and cefotaxime (30 µg) were used for screening for ESBLs. Mueller- Hinton Agar (MHA) were prepared and inoculated with the test organism (turbidity corresponding to 0.5 McFarland's standard) to form a lawn culture. The above discs were applied on the surface of the agar. The plates were incubated at 37 º C overnight and sensitive pattern and resistant pattern were recorded by reading the zone diameter of the test organism. If a zone diameter of ≤ 22mm for Ceftazidime and ≤27 mm for cefotaxime was recorded these strain were considered "Suspicious" for ESBL production [10, 17].

Double Disk Approximation Test (DDAT)

Bacterial suspension equivalent to 0.5 McFarland standards turbidity for testing ESBL production test were prepared. A sterile swab was dipped into standardized inoculum and the soaked swab was rotated against the upper inside wall of the tube to express excess fluid. The entire surface of the MHA was swabbed to form a lawn culture and the inoculum was allowed to dry for a minute with lid in place. With sterile forceps, Ceftazidime disk was placed on the agar plate near the centre giving a centre to centre distance of 15 mm Ceftazidime/clavulanic acid (30µg/10µg). The plates were inverted and incubated at 37ºC for 16-18 hours. Each plate was examined for enhancement of zone of inhibition for ceftazidime disk at the side facing Ceftazidime/clavulanic acid disk. If the strain was an ESBL producer, then the zone around ceftazidime disk was extended towards Ceftazidime/clavulanic acid disk. ATCC *Escherichia coli* -

ISSN: 0975-8585

25922 were used as negative control and ATCC *K. pneumoniae* -700603 was used as positive control [17-21].

Detection of Metallo β-Lactamases by Imipenem-EDTA-Double Disk Synergy Test

The IMP-EDTA combined disk test was performed as described by Yong *et al*. Test organisms were inoculated on to plates with MHA as recommended by the CLSI guidelines. Two 10 µg imipenem disks (Hi Media, Mumbai India) were placed on the plate, and appropriate amounts of 10 µL of 0.5 M EDTA solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of incubation at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as MBL positive [10, 17, 20].

RESULTS AND OBSERVATIONS

During the 4-years study period (2008 to 2011), there were 3180 samples were received from septicaemic patients from various wards. Of these, 1657(52.10%) were culture positive. Isolated organisms included Gram negative bacteria (GNB) 894(53.95%), Gram positive cocci (GPC) 736(44.41%), fungus 27(1.62%).GNB were more frequently isolated than GPC. (Table1)

Table 1. Year wise distribution of Blood culture isolates from septicaemia patients.

YEAR	TOTAL SAMPLE RECEIVED	CULTURE POSITIVITY	GRAM-NEGATIVE BACTERIA	GRAM-POSITIVE BACTERIA	FUGUS
2008	503	263	170	90	3
2009	583	290	206	79	5
2010	844	503	213	282	8
2011	1250	601	305	285	11
Total	3180	1657(52.10%)	894(53.95%)	736(44.41%)	27(1.62%)

Table 2. Year wise distribution of gram positive cocci from culture positive cases.

Organism isolated / Year	*M.S.S.A.*	*M.R.S.A.*	*C.O.N.S.*	*STREPTOCOCUUS SPP.*
2008	53	26	8	3
2009	35	20	16	8
2010	143	72	30	37
2011	134	80	39	32
TOTAL	365(49.59%)	198(26.90%)	93(12.63%)	80(10.88%)

Common isolates among GPC were MSSA 365(49.59%) followed by MRSA 198(26.90%).Other pathogens were CONS 93(12.63%) and Streptococcus spp. 80(10.88%) (Fig.1). The most common GNB implicated were *Klebseilla spp*. 257(28.74%), *Acinetobacter spp.* 179(20.%), *E. coli* 153 (17.11%*), Citrobacter spp.*121 (13.53%) *followed by Enterobacter spp. 108(12%).* While *Pseudomonas spp. Salmonella spp. Proteus spp*. were isolated in lesser

ISSN: 0975-8585

numbers. Year wise distribution of Gram negative bacilli showed in (table 3). Distribution of GNB and GPC in various wards in (table 4).

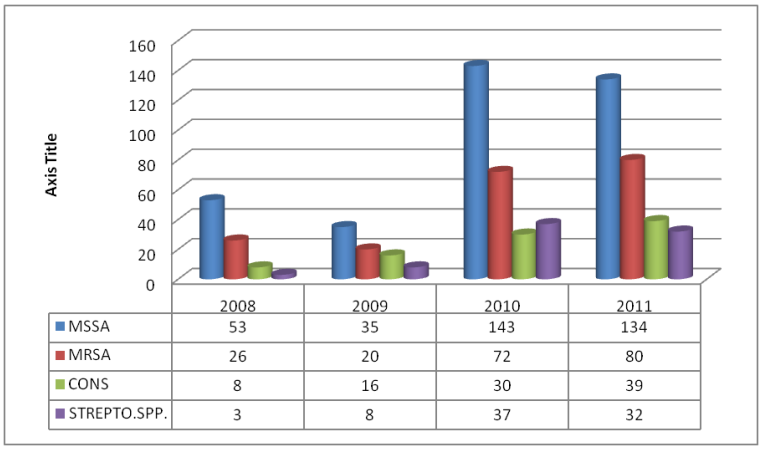

Figure 1 Year wise Distribution of Gram Positive Cocci

Table 3. Year wise distribution of Gram negative bacilli.

ORGANISM ISOLATED PER YEAR	2008 N=170	2009 N=206	2010 N=213	2011 N=305	Total N= 894	ESBL N= 259 (28.97%)	MBL N= 49 (5.48%)
E. coli	36	40	33	44	153	53	08
Klebseilla spp.	45	63	67	82	257	62	17
Acinetobacter spp.	29	37	44	69	179	64	10
Citrobacter spp.	19	15	31	56	121	44	06
Enterobacter spp.	23	30	25	30	108	20	01
Pseudomonas spp.	06	12	04	07	29	12	05
Salmonella spp.	04	03	04	03	14	02	00
Proteus spp.	06	03	01	09	19	01	01
Others	02	03	04	05	14	01	01
Total							

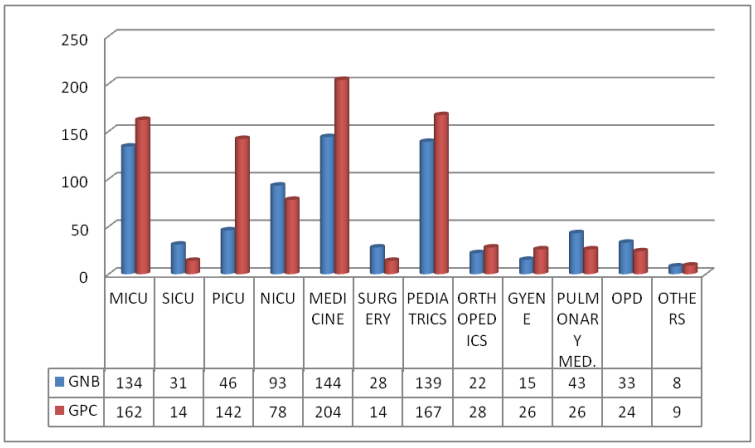

Figure 2 Distribution of GNB and GPC in various wards.

ISSN: 0975-8585

Table 4. Distribution of GNB and GPC in various wards.

WARDS	GRAM POSITIVE COCCI	GRAM NEGATIVE BACILLI	TOTAL (N=1657)
MICU	134	162	296(17.86%)
SICU	31	14	45
PICU	46	142	188
NICU	93	78	171
MEDICINE	144	204	348
SURGERY	28	14	42
PEDIATRICS	139	167	306
ORTHOPEDICS	22	28	50
GYENAC	15	26	41
PULMONARY MEDICINE	43	26	69
OPD	33	24	57
OTHERS	08	09	17
TOTAL	736	894	1630

*Ceftazidime + Clavulanic acid disc susceptibility testing were done on only Ceftazidime resistance strains. Imipenem + EDTA disc susceptibility testing were done on only Imipenem resistant strains.

Maximum culture positive isolates were received from medicine ward followed by pediatric ward. Total of 700 culture positive samples were received from four ICUs i.e. MICU 269(38.42%), PICU 188(26.85%), NICU 171(24.42%), SICU 45(6.42%). Neonatal septicemias were observed in significant number. Distribution of GNB and GPC in various wards (Fig.2). The results of antibiotic resistance pattern among GBN isolates are shown in table no.4. of the 894 culture positive GNBs, 259 (28.97%) isolates were ESBL producers, while 14(1.56%) were MBL producers. Highest susceptibility were observed in Imipenem amongst antibiotic tested. Reduced susceptibility were observed in tetracycline (29.08%), amoxicillin (32.99%), Amikacin (35.23%), cefotaxime (39.14%). Moderate susceptibility were observed in ciprofloxacin (59.39%) ceftazidime (65.88%), co-trimaxazole (45.19%). Most of *Klebseilla pneumonia* and *E. coli* were resistant to Ceftazidime, ampicillin and tetracycline. Antimicrobial susceptibility pattern of GNB isolates (table 4).

DISCUSSION

This study showed the prevalence of septicaemia about 52.10%. Of the total 894 GNB, *K. pneumonia* 257(28.74%), *Acinetobacter spp*. 179(20.02%) and *E. coli* 153(17.11%) were the most common from blood samples. There management was complicated by their production of ESBLs and appear to be a result of heavy use of broad spectrum cephalosporins. *Acinetobacter baumanii* is an opportunistic pathogen commonly associated to nosocomial outbreaks worldwide because of its ability to survive harsh environmental conditions such as desiccations, disinfecting solutions and temperature variations [22-25]. We found *Acinetobacter spp.* commonly from PICU and MICU and annually its frequency of isolation is increasing in order. Isolation of *Acinetobacter spp*. was observed significantly in hospitalized patients, so most probably associated with long duration of stay in hospital. However, limitation of this study is that we did not collect data to deliberate severity of illness to confirm this suspicion. Present

study demonstrated 171 (10.31%) culture positivity from NICU. GNB septicaemias were predominant as compared to GPC septicemia. No significant differences were seen in positive blood culture results was observed between early and late onset sepsis in neonatal septicemia. Worldwide 4 to 5 million infants die during the first month of life in very low birth weight (VLBW) and 98% of these deaths occur in underdeveloped countries [26,27]. About 28.5% neonates were died and increased mortality was seen in sepsis associated with ESBL and MRSA isolates. We found Similar to that observed in other studies in East Africa region by Mugalu J *et. al* and Singh Sa *et. al* [28].

MDR GNB posses the grave threat of infections that are untreatable. The incidence of carbapenem resistant strains is rising worldwide, reflecting regional, geographical and institutional differences. In the present study about 259(28.97%) were ESBL producers and 14(1.56%) were MBL among all GNBs. This finding is consistent with reports from other developing countries such as Tanzania [22], Uganda [23], Nigeria [24] but higher than those reported in developed countries [25]. About 736 (44.41%) were found to be GPCs and about 365 (49.59%) were *Methicillin Sensitive Staphylococcus aureus* (MSSA) and 198 (26.90%) were *Methicillin resistance Staphylococcus aureus* (MRSA). Of the total GPCs, 93 (12.63%) and 80 (10.88%) were *Coagulase negative Staphylococcus aureus* (CONS) and *Streptococcus spp.* respectively. CONS are common cause of nosocomial infection and the most common cause of blood stream infections in the intensive care setting. We recorded CONS septicemia in patients who were very low birth weight (VLBW) neonates, immunocompromised (lymphoma, leukemia, post-bone marrow transplantation), and have indwelling intravascular devices, ventricular shunts, peritoneal catheter. CONS infections were the most common infections causing late-onset sepsis (>72 hrs. of age) in infants born in VLBW. Establishing the diagnosis of CONS sepsis can be complex and difficulty exists in differentiating infection from contamination. We have used neonatal definitions for CONS infections involve one positive culture with clinical sign or symptoms or one positive culture with elevated inflammatory markers. The present study showed excellent susceptibility to Vacomycine and Linezolid. Linezolid, an oxazolidinone, is a promising alternative to vancomycin in the treatment of resistant staphylococcal infections. In vitro, linezolid, even at sub inhibitory concentrations, inhibited adhesion of *S. epidermidis* to polystyrene wells, whereas vancomycin had similar effects only at supra-inhibitory concentrations. Majority of the neonates with MDR GNB died within 72 hrs. of initiations of antimicrobials. Relatively good survival was confirmed in neonates with sensitive organisms and more than 85% of them improved after 72% of treatment. Similar findings were reported by Bloomberg B. *et. al.(*2005) and Neema Kayange *et.al* [25-27].

No Drug has reached advanced stages of development for the treatment of infections due to MDR-GNB such as *Acinetobacter spp., Pseudomonas spp., and Klebseilla spp*. Most of *K. pneumonia* and *E. coli* were resistant to ampicillin, Gentamicin, sulpha drugs and third generation cephalosporins. The discovery of new scaffolds should be a priority attending new drugs for such bacteria. Unfortunately, antibiotic discovery and development are on decline. The unfavorable economics of antibiotic development is related to their limited use compared to prescriptions for chronic illness. Also newly approved drugs may often be restricted to the treatment of serious illness. This has lead to pharmaceutical companies decreasing interest in

new drug development. Rediscovering new applications for older antibiotics represents one of the alternative ways of managing MDR organisms. Polymyxins, known since 1947 and represented mostly by polymixin B and polymyxin E (colistin), have recently gained a principal role in the treatment of the most problematic MDR GNB infections. In the present study majority of GNB isolates were susceptibile to ceftazidime/tazobactam and imipenem, tigecyeline, polymyxin-B [27-34].

Our study has certain limitations that this is retrospective analysis and performed at single tertiary care hospital so relatively small cohort of patients and was underpowered to detect a clinically significant difference in outcome. We could not performed molecular techniques to characterization of MDR GNB strains to know their genetically similarity and diversity.

CONCLUSION

The expeditious detection and identification of blood-borne pathogens is one of the most important functions of the microbiology laboratory. Positive blood culture may help to provide a clinical diagnosis. Decision regarding empirical treatment of septicaemia must be based on a sound knowledge of the local distribution of the pathogens with susceptibility pattern. Awareness of changing pattern of MDR pathogens and understating of associated risk factors can develop the effectiveness of empirical treatment protocols. In this perception, close collaboration between physicians, clinical microbiologist and infectious-disease authority should produce significant positive effects.

ACKNOWLEDGEMENT

We would like to extend our thanks to management of the D. Y. Patil University Pune and special thanks to Dr. P.D. Patil and Mrs. Bhagyashritai Patil for their help, support and encouragement for research work. We acknowledge assistance provided by Miss. Aster, Miss. Anuradha, Miss. Prayadna at different stages of this study.

REFERENCES

[1] Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP,et al. 2001; 39: 2206-12.
[2] Bradford PA. 2001; 14: 933-51.
[3] Wayne PA. 2008; M100-S18.
[4] Babini GS, Livermore DM. 2000; 45: 183-9.
[5] Moland ES, BlacK JA, Ourada J, Reisbig MD, Hanson ND, Thomson KS. 2002; 46: 3837-42.
[6] Siegel JD, Rhinehart E, Jackson M, Chiarello L (2006) Available from:http://www.cdc.gov/ncidod/dhqp/pdf/ar/MDRO Guideline2006.pdf.[accessed on 2009 feb 23]
[7] Anderson DJ, J Engemann, LJ Harrell, Y Carmeli, LB Reller, and KS Kaye. 2006; 5: 1715-20.

[8] Schwaber MJ, S Navon-Venezia, KS Kaye, R Ben-Ami, D Schwartz, Y Carmeli. 2006; 50: 1257-62.
[9] Das A, Ray P, Garg R, Kaur B. Proceeding of the Silver Jubilee Conference. All India Institute of Medical Sciences, 2001, New Delhi.
[10] Bush K, Jacoby G.A, Medeiros AA. 1995; 39: 1211-33.
[11] M Purva, K Arti, D Bimal, D Benu. 2002; 115: 153-57.
[12] Shukla I. 2004; 22: 87-91.
[13] Babypadmini S, Appalaraju B. 2004; 22: 172-4.
[14] Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing. In 18th informational supplement M100-S18.2009 Wayne, CLSI; 2008.
[15] Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infection. Mosby, 1996: A-1-A-20.
[16] Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Indian J Med Res 2004; 120: 172-4.
[17] Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. 1997: 110-45.
[18] Livermore DM. 1995; 8: 557-84.
[19] Bush K, Jacoby G.A, Medeiros AA. 1995; 39: 1211-33.
[20] Bloomberg B, Jureen R, Manji KP,Tamim BS, Mwakagile DSM, Urassa WK, Fataki M, Msangi V, Tellevik MG, Maselle SY, Langeland N. 2005; 43(2): 745-49.
[21] EL-Karsh T, Tawfik AF, AL Shammary F, Al-Salah S , Kambal AM. 1995; 7: 509-14.
[22] Schwaber MJ,Nsvon-Venezia S,Kaye KS,Ben-Ami R, Schwartz D. 2006; 50: 1257-62.
[23] Lauplant KB, Gregson DB, Church DL, Ross T, Pitout JD. 2008; 14: 1041-47.
[24] Irebu KC, Elegba OY, Babaniyi IB. 2006; 6(3): 151-54.
[25] Yu Y, Zhou W, Chen Y, Ding Y, Ma Y. 2002; 115: 1479-82.
[26] Tumbarello M, Sanguinetti M, Montuori E, Trecarichi ME. 2007; 51: 1987-94.
[27] Weber MW, Carlin JB, Gatchalian S, Lehmann D, Muhe L. 2003; 22(8): 711.
[28] Monga K, Fernandez A, Deodhar L. Indian J Pediatr 1986; 53: 505-8.
[29] Apisarnthanarak A, Kirantisin P, Mundy LM. 2008; 29: 671-74.
[30] Kim BN, Woo JH, Kim MN, Ryu J, Kim YS. 2002; 52: 99-106.
[31] Schwaber MJ, Carmeli Y. 2007; 60: 913-20.
[32] Avasthi TS, Kumar N, Baddam R, Hussain A, Nandanwar N, Jadhav S and Ahmed N. 2011; 193: 4272-3.
[33] Jadhav Savita, A. Hussain, S Devi, A Kumar, S Parveen, N Gandham, LH Wieler, C Ewers, and N Ahmed. 2011; 6: e18063.
[34] Hansonita JB, Agarwal V, Pathak AA, Saoji AM. 1997; 105: 160-5.