

ORIGINAL ARTICLE

Gram negative organisms in community acquired respiratory tract infections

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ABSTRACT

Objective: Respiratory tract infections (RTIs) are amongst the most common conditions encountered in our hospitals. The objectives of the study were to determine the frequency and antibiotic susceptibility pattern of various Gram negative bacteria (GNB) isolated from sputum samples of patients with RTIs.

Methods: A descriptive, cross sectional study was carried out at the department of Microbiology, Army Medical College. Patients were diagnosed as suffering from RTIs on the basis of clinical features, radiological and laboratory findings. Sputum samples from all admitted and outdoor patients that presented to the hospital with RTIs were collected using sterile plastic containers. Those that satisfied the criteria for inclusion were accepted and processed according to routine microbiological procedures. The cultures were identified and susceptibility patterns of the organisms noted.

Results: Fifteen hundred and ninety six sputum samples were processed of which 549 (34.4%) yielded a positive pathogenic organism on culture with 327 (59.6%) yielding GNB. Of these *Pseudomonas aeruginosa* 105 (32.1%) was the most frequently isolated organism followed by *Haemophilus influenzae* 62 (19.0%), *Klebsiella pneumoniae* 54 (16.5%), *Acinetobacter* 46 (14.1%) and *E. coli* 41 (12.5%). Piperacillin/tazobactam, meropenem and imipenem were among the most effective antibiotics.

Conclusion: Gram negative bacteria are becoming an increasing cause of RTIs and hence demand an increased prudence and clinical insight in the treating clinician to keep on a lookout for these organisms as the causative agent of RTIs. J Microbiol Infect Dis 2013; 3(1): 8-11

Key words: Antibiotics, resistance, Gram negative bacteria, respiratory tract infection

Solunum yolu enfeksiyonu olan hastaların balgam örneklerinin gram negatif mikroorganizmaları

ÖZET

Amaç: Solunum yolu enfeksiyonları (SYE) hastanemizde karşılaşılan en yaygın durumlar arasındadır. Çalışmanın amacı balgam örneklerinden izole edilen çeşitli Gram negatif bakterilerin (GNB) sıklığını, antibiyotik duyarlılık paternini belirlemektir.

Yöntemler: Tanımlayıcı, kesitsel çalışma Ordu Tıp Koleji Mikrobiyoloji Bölümü'nde yürütüldü. Solunum yolu enfeksiyonu şüphesi olan hastalara klinik özellikleri, radyolojik ve laboratuvar bulguları esas alınarak SYE tanısı kondu. Hastanemize başvuran hastaların ve ayaktan hastaların tümünün balgam örnekleri steril plastik kaplar kullanılarak toplandı. Bunlardan inklüzyon kriterlerini karşılayanlar rutin mikrobiyolojik usullere göre kabul edildi ve işleme alındı. Kültürleri yapıldı ve organizmaların duyarlılık paternleri kaydedildi.

Bulgular: Kültür pozitif olan 549 (% 34,4) balgam örneğinin GNB üreyen 327 (% 59,6)'si işleme alındı. Bunlardan en sık izole edilen mikroorganizma olan *Pseudomonas aeruginosa*'yı 105 (% 32,1); *Haemophilus influenzae* 62 (% 19,0), *Klebsiella pneumoniae* 54 (% 16,5), *Acinetobacter* 46 (% 14,1) ve *E. coli* 41 (% 12,5) takip ediyordu. Piperasilin / tazobaktam, meropenem ve imipenem en etkili antibiyotikler arasındaydı.

Sonuç: Gram negatif bakteriler SYE'nin giderek artan bir nedeni haline gelmektedir ve bu nedenle klinisyenin bu organizmaların SYE'lerinde etken ajan olabileceği konusunda uyanık olması ve tedavideki farkındalığın artması ve klinik içgörüsü istenmektedir.

Anahtar Kelimeler: Antibiyotikler, direnç, Gram negatif bakteriler, solunum yolu enfeksiyonu

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INTRODUCTION

Respiratory tract infections (RTIs) are some of the most important conditions affecting patients worldwide. While the upper respiratory tract infections (URTI) are associated with high morbidity, the lower respiratory tract infections are linked to high mortality and are considered a greater risk to patients' health.¹ Despite the widespread use of antimicrobial agents, pneumonia, a form of this condition, remains an important cause of morbidity and mortality for the non-hospitalized adults. These infections may also result in exacerbations of chronic obstructive pulmonary disease (COPD), the latter being recognized as an important cause of the high morbidity and mortality.²

Viral infections are considered to be the cause in most cases of respiratory tract infections.³ However; recent studies have correlated COPD exacerbation and pneumonias with the overgrowth of the bacterial load or with the acquisition of a new strain of pathogenic bacteria. In ambulatory patients, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* are the three major pathogens isolated in COPD exacerbations.⁴ *Pseudomonas aeruginosa*, even though less common, has been associated with acute exacerbation of COPD. The organism was more frequently isolated in patients that had a history of smoking, steroid therapy and a higher frequency of hospital admission in the preceding months and years. Other characteristics of patients hospitalized with such exacerbations included old age, male gender (due to greater association with smoking) and obesity.⁴

The frequencies of pathogens responsible for respiratory tract infections are changing. It has become necessary to determine the frequency of various organisms prevalent in different regions of the world. It has also become imperative for clinicians to establish a specific etiologic diagnosis before initiating therapy and to consider the diagnostic possibilities and treat with antimicrobial agents that are effective against the most likely pathogens.¹ Likewise, despite the enormous impact of COPD, the treatment options currently available for prevention and management of exacerbations are inadequate and new therapies are urgently needed.⁵

This study was, therefore, carried out to determine the frequency and nature of organisms prevalent in our environment. This would enable us to be better prepared against these pathogens in the form of pre-determined and effective empirical antibiotic regimens.

METHODS

A descriptive, cross sectional study was carried out in the department of Microbiology, Army Medical College, National University of Sciences and Technology, Pakistan, on the sputum samples of patients presenting to the Military Hospital, Rawalpindi, Pakistan. The study spanned a period of one and a half years i.e. from May 2009 to February 2011. The inclusion criteria comprised all patients that presented to the hospital with respiratory tract infections in the time frame of the study. These included both upper and lower RTIs diagnosed in both out door and newly hospital admitted patients. Patients were identified on the basis of clinical features including cough, sputum production and fever, along with associated radiological and laboratory findings that suggested an underlying respiratory tract infection. The aim was to determine the frequency of Gram negative organisms isolated from the sputum samples sent from the hospital and to find the most effective antibiotics against the most frequent organisms. An informed consent form and institutional review board approval was received from the Army Medical College/ Military Hospital Pakistan review board. Samples that were included in the study were acquired from both outdoor and newly admitted hospital patients that presented with such respiratory tract infections. In the latter group, samples were collected within 24-48 hours of admission. Based on these inclusion criteria, the organisms isolated were considered as community acquired.

Sputum samples were collected in sterile plastic containers and then transferred to the microbiology lab of Army Medical College. Only those samples with adequate amount of sputum were accepted. Those that contained inadequate amount of sputum for analysis or that contained only saliva were excluded. All samples were first Gram stained and examined under microscope to divide them into Gram positive and Gram negative groups. They were then dealt with according to standard microbiological procedures and were grown on respective culture media whereby cultures yielding growth of different organisms were recorded and analyzed. Those organisms that resembled the normal throat flora were excluded from the study. Only those that resulted in growth of adequate quantities of colonies were accepted and included in the study. All isolates, irrespective of the age and sex of the patient, were included in the study. Data was recorded and analyzed using "Microsoft Excel 2010". The number of Gram negative bacteria isolated was determined and their percentage as a total of all the organisms isolated was found. Individual frequen-

cies of different organisms in the above mentioned group were determined along with most effective antibiotics against each one.

RESULTS

A total of 1606 sputum samples were submitted during the study period. Of these, 549 (34.4%) samples yielded a positive pathogenic organism on their subsequent inoculation on culture media. Gram negative bacteria (GNB) were the most frequently isolated organisms (Figure 1). Of these, *P. aeruginosa* was on the top with 105 (32.1%) organisms isolated followed by *H. influenzae*, *K. pneumoniae*, *Acinetobacter* and *E. coli* with 62 (19.0%), 54 (16.5%), 46 (14.1%) and 41 (12.5%), respectively. Only 11 (3.4%) of cultures yielded growth of *Enterobacter* while seven (2.1%) isolates yielded growth *M. cattarrhalis* and only a single culture yielded *Serratia marascens*. The next organism most frequently isolated after the GNB was *Candida albicans* with 179 (32.6%) positive isolates. The least frequent were the Gram positive bacteria and *Aspergillus*. There were only 39 (7.1%) isolates that gave a positive Gram positive result. Of these, *S. aureus* was the most frequently recovered with 20 (51.3%) positive cultures, followed by Streptococci with 14 (35.9%) and coagulase negative Staphylococci with five (12.8%) isolates.

Against *P. aeruginosa*, the four most effective drugs were piperacillin/tazobactam with all 65 (100%) isolates tested being sensitive, meropenem with 60 out of 62 (96.8%) isolates tested sensitive, cefoperazone 49/52 (94.2%) and imipenem with 26/27 (96.3%) sensitive isolates respectively. The moderately effective antibiotics included ceftazidime with 43/47 (91.5%), amikacin 57/63 (90.5%), co-amoxiclavate 8/9 (88.9%), gentamicin 62/72 (86.1%) and ciprofloxacin with 53/63 (84.1%) sensitive organisms. Ampicillin with 14 out of 56 (25%) sensitive isolates among those tested and levofloxacin with 20 out of 32 (62.5%) were the least effective.

The most effective antibiotics against *H. influenzae* were aztreonam, imipenem and piperacillin / tazobactam with 100% sensitive organisms (7, 8 and 41 respectively). Fifty one out of 56 (98.1%) organisms tested for ceftriaxone were sensitive to it while 45/47 (95.7%) were to co-amoxiclavate, 36/37 (97.3%) to meropenem and 29/30 (96.7%) to gentamicin. Ampicillin and levofloxacin again showed decreased efficacy with 40/58 (69%) and 42/56 (75%) organisms sensitive, along with penicillin with 29/58 (50%), erythromycin 13/42 (31%), cotrimoxazole 11/52 (21.2%) and tetracycline 41/61 (67.2%). Imipenem, meropenem and amikacin were the most ef-

fective antibiotics against *E. coli* with 11/11 (100%), 22/25 (88%) and 23/25 (92%) organisms sensitive respectively while co-amoxiclavate, ciprofloxacin and ampicillin were the least effective with 8/35 (22.9%), 8/35 (22.9%) and 2/35 (5.7%) organisms.

DISCUSSION

Our study showed that of 1606 samples, 549 (34.4%) gave a positive pathogenic organism on culture of the specimen. One study showed that sputum culture were positive in 55.5% of the samples analyzed while another carried out by Edirisinghe showed that the yield by sputum culture was 57.6%.^{6,2} The lower yield from our study could be attributed to higher frequency of viral infections in the region which require other methods of detection than the ones carried out for bacteria. Another explanation could be the use of empirical antibiotics in these patients resulting in pre-diagnosis eradication of the organisms.² However, sputum culture and Gram staining are neither specific nor sensitive as diagnostic tools. The number of pathogens involved in respiratory tract infections is large constituting an enormous challenge for diagnostic microbiology. In general, in only 50% of cases is an etiologic agent detected.⁷ Additionally, the URT, with its commensal flora, acts both as a defense mechanism and as a primary site for LRTI, creating tremendous diagnostic challenges. Any bacteriological examination of non-sterile respiratory specimens must indeed distinguish between organisms infecting the LRT and organisms colonizing the rhino pharynx.⁷ Likewise, to be of value for microbiological diagnosis, sputum specimens must be representative of lower respiratory secretions.⁷ The values determined in other studies are much higher than the results we determined from our study. This discrepancy could also be because of higher frequency of viral etiology in our region, as stated earlier, or may be because of less sensitive techniques and equipment used due to lesser resources in our set up.

The frequency of Gram negative bacteria was 59.6%, closely matching that of 61.9% determined by Edirisinghe and 68.72% by Wang.^{2,8} Another study showed that in every tenth patient with community acquired pneumonia, etiology due to Gram negative bacteria had to be considered.⁹ *P. aeruginosa* was the most commonly isolated organism of the Gram negative bacteria (32.1%). The same results but with lower frequency, 18% and 16.88%, were obtained by the above two researches in their studies respectively. Another study carried out by Francisco Arancibia found that the organism accounted for 65% of the Gram negative bacteria.⁹ The different percentages in these studies could be

explained by varying numbers of other organisms of the group, depending upon the region, affecting the relative frequency of *Pseudomonas aeruginosa* among other Gram negative bacteria. Other pathogens isolated from our study include *H. influenzae*, *K. pneumoniae*, *Acinetobacter* and *E. coli* 62 (19.0%), 54 (16.5%), 46 (14.1 %) and 41 (12.5%) organisms respectively. Investigators have determined varying percentages of different Gram negative organisms in various studies. Edirisinghe found that *Klebsiella* was the most common organism isolated, followed by *P. aeruginosa* and *M. catarrhalis* while Wang found *P. aeruginosa* (16.9%) to be more frequently isolated followed by *K. pneumoniae* (10.8%), *E. coli* (10.7%) and fungi (10.6%).^{2,8} In another study, *H. influenzae* was shown to be the most frequent organism again followed by *P. aeruginosa*, *S. pneumoniae* and *M. catarrhalis*. The same study also showed that the former two were associated with more severe respiratory compromise in the form of worsened Forced Expiratory Volume (1 second) and were more likely to be the causative organisms rather than commensals. Gram positive organisms, including *Staphylococci* and *Pneumococci*, were in far less numbers as compared to the other organisms.⁸ The latter finding was also in concordance to the frequency of Gram positive bacteria determined by our study i.e. only 7.1% were isolated. These discrepancies can be accounted for the various regional differences in terms of diet, immunity and associated co-morbid conditions, thus favoring the flourishing of some organisms over others.

The most effective antibiotic against the Gram negative bacteria was imipenem with 96.3% sensitivity against *Pseudomonas aeruginosa* and 100% against *H. influenzae* and *E. coli*. This is in concordance with the study by Karamat where 91% sensitivity was determined for *Pseudomonas aeruginosa* and 100% for latter two bacteria.¹⁰

The main limitation of our study was that it could not be adequately determined if the organism isolated was truly a pathogenic one or a commensal. However, despite not having direct evidence of them causing the infection, *P. aeruginosa* and *H. influenzae* are associated with more severe disease and more likely to be causative organisms instead of commensals. The higher frequency of these organisms in our setup means that our population is more susceptible to them. This fact may be attributed to hardy nature of *P. aeruginosa* with associated risk factors prevalent in our region including warm and humid weather with general lack of hygiene in the local population. Another limitation, due to financial constraints, is the inability to determine the resistance pattern of the fungal organisms isolated. The

frequency of fungal infections is high and the detection of antifungal resistance would be useful for treatment of community-acquired fungal diseases. In addition, there is a general lack of literature on the risk factors and demographic properties of patients which predispose individuals to acute exacerbations of COPD as a consequence of pneumonia or associated infections.

In conclusion, Gram negative bacteria are the more common organisms isolated from sputum samples in patients of upper and lower respiratory tract infections in our setup. The most common organism is *P. aeruginosa* followed by *H. influenzae* and *K. pneumoniae* with the most effective antibiotics being of piperacillin/tazobactam, meropenem, imipenem, and aztreonam. In light of the above stated limitations and conclusions, physicians in our region must remain vigilant and keep these facts in mind while prescribing antibiotics to patients of respiratory tract infections. Further studies should be undertaken to separately identify the most common organisms associated with upper and lower respiratory tract infections.

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