Jpn. J. Infect. Dis., 58, 50-52, 2005

Short Communication

Major Bacteria of Community-Acquired Respiratory Tract Infections in Turkey

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(Received August 2, 2004. Accepted October 22, 2004)

SUMMARY: To determine the bacterial etiology of lower respiratory tract infections (LRTIs) in Turkey, quantitative cultures of sputum were carried out. The major pathogens for LTRIs were found to be *Haemophilus influenzae*, followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*. Only 6.1% of the *H. inlfuenzae* and all strains of *M. catarrhalis* were β-lactamase producers. An E-test showed that 31.2% of the *S. pneumoniae* strains had an intermediate resistance to penicillin, and the remaining strains were susceptible; no fully resistant strains were detected.

From the perspective of the world community, acquired lower respiratory tract infections (LRTIs) are an important cause of morbidity and mortality for all age groups. Each year, approximately 7 million people die as a direct consequence of acute and chronic respiratory infection (1). LRTIs are very common, with an incidence in the world population of 40 - 50 per 1,000 (2,3). Since the etiological agents of LRTIs cannot be determined clinically, microbiological investigation is critical for both treatment and epidemiological purposes. A quantitative sputum culture is a reliable and non-invasive procedure used for the determination of causative bacteria (4,5). Although a sputum culture has the advantage of high sensitivity, routine laboratories are not able to perform this test for various reasons. Therefore, antimicrobial therapy for LRTIs is frequently empirical, generally presumptive and instituted before the etiology of the specific disease is known. Empirical antimicrobial choice is complicated by the increasing prevalence of resistance of some of the common LRTI pathogens to antibiotics (6). In order to select appropriate antibiotics for empirical therapy, epidemiological studies are critical to identify the actual causative microorganism and to determine whether it is susceptible to antibiotics. To save lives and to reduce mortality with proper treatment, there is no alternative other than to obtain this information for each country.

Information in this regard is scarce in Turkey. The present study represents a joint effort between respirologists, infectious disease specialists, and microbiologists to introduce a platform for continued future surveillance. To determine the bacterial etiology of LRTIs, their resistance to antibiotics and β-lactamase production was determined in two major hospitals in Ankara, Turkey, in a prospective study.

Between September of 2002 and April of 2003, sputum samples were collected from adult LRTI patients who were attending the inpatient and outpatient clinics of the Ataturk Chest Disease and Chest Surgery Central Education and Research Hospital, and Ibni Sina Hospital, Ankara.

Specimens consisting of saliva were examined by macroscopic observation. Gram staining was then performed and specimens containing more than 25 polymorphonuclear neutrophils (PMNs) and fewer than 10 epithelial cells per low-power field were included in the study (7). The state of PMNs, and PMNs with intracellular bacteria under an oil-immersion lens were also assessed for each sample. Samples showing more than 10 epithelial cells per field with more than 25 PMNs were also included if predominating intracellular bacteria were observed.

Quantitative cultures of sputum and determination of the significant respiratory bacterial flora were carried out following a previously described method (4) with the following modification: after a serial ten-fold dilution of the homogenized sputum, the samples were inoculated on GC chocolate agar plates and blood agar plates containing 5% human blood.

The antibiotic susceptibility of *Streptococcus pneumoniae* strains to penicillin was determined by E-test (AB Biodisc, Solna, Sweden). MIC breakpoints were determined according to the recommendations of the National Committee for Clinical Laboratory Standards (8). β-lactamase production was determined for *Moraxella catarrhalis* and *Haemophilus influenzae* strains using a Cefinase disc following the instructions of the manufacturer (Nitrocefin disc; Becton, Dickinson and Co., Sparks, Md., USA).

By observing the status of PMNs and the epithelial cells in a Gram-stained smear, 165 (52.0%) of 317 collected sputum specimens were determined to be suitable for culture. These samples represented 103 (62.4%) male and 62 (37.6%) female patients whose median age was 57 (17 - 102) years. Using a cut-off value of 10^7 cfu/ml, significant growth was found in 98 (59.4%) samples, which yielded clinically significant pathogens that could be implicated as causative agents. Single and mixed pathogens were isolated from 87 (88.8%) and 11 (11.2%) samples, respectively (Table 1).

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Table 1. Isolated causative bacteria identified by quantitative culture of sputum using a cut-off value of 10^7 cfu/ml

Isolated pathogenic bacteria	Number of cases (%) (n = 98)
Single pathogen	
Haemophilus influenzae	44 (44.9)
Streptococcus pneumoniae	25 (25.5)
Moraxella catarrhalis	12 (12.2)
Pseudomonas aeruginosa	3 (3.1)
Klebsiella spp.	1 (1)
Haemophilus parainfluenzae	1 (1)
Staphylococcus aureus	1 (1)
Multiple pathogens	
H. influenzae + *S. pneumoniae*	6 (6.1)
M. catarrhalis + *S. pneumoniae*	2 (2)
M. catarrhalis + *H. influenzae*	1 (1)
S. pneumoniae + *H. parainfluenzae*	2 (2)
Total	98 (100)

The most common single pathogen was *H. influenzae*, which was isolated from 44 patients (44.9%), followed by *S. pneumoniae* (25 patients, 25.5%) and *M. catarrhalis* (12 patients, 12.2%). Both *H. influenzae* and *S. pneumoniae* were isolated from 6 samples (6.1%), *M. catarrhalis* and *S. pneumoniae* from 2 samples (2.0 %), *M. catarrhalis* and *H. influenzae* from 1 sample (1. 0%), and *S. pneumoniae* and *Haemophilus parainfluenzae* from 2 samples (2.0%) (Table 1).

Complete clinical information was obtained from 49 of 98 patients. Fifteen (30.6%) of these patients were diagnosed with community-acquired pneumonia, 26 (53.1%) with acute exacerbation of chronic obstructive pulmonary disease (COPD) and 8 (16.3 %) with chronic pulmonary disease with infection. *S. pneumoniae* was isolated in similar proportions from all three disease categories. More than 50% of the isolated *H. influenzae* and *M. catarrhalis* strains were from patients with COPD, followed by patients with pneumonia (more than 30%). A total of 18 (36.7%) of these 49 patients were under antibiotic therapy and the median time of use for an antibiotic was 3 days. The most commonly used antibiotics were ampicillin-sulbactam 10 (55.6%), clarithromycin 4 (22.2%), ciprofloxacin 3 (16.7%) and ampicillin-sulbactam plus clarithromycin 1 (5.5%). There was no significant difference in the isolation of mixed pathogens between patients under and not under antibiotic treatment (11.1% versus 12.9%).

All of the 15 strains of *M. catarrhalis* were β-lactamase positive. A total of 49 strains of *H. influenzae* strains were available for β-lactamase testing, and 3 (6.1%) were found to be β-lactamase positive. A total of 32 strains of *S. pneumoniae* were available for penicillin susceptibility testing; 10 (31.2%) showed intermediate resistance, and the remaining 22 (68.8%) strains were susceptible. No fully resistant strains were detected.

This first prospective study in Turkey for the detection of pathogens by a quantitative culture of sputum revealed that, as in other parts of the world (4), the major pathogens of community-acquired LTRIs are *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis*.

We found that significant numbers of LRTIs are caused by multiple pathogens. The identification of multiple pathogens is very important for treatment strategies and to avoid a false impression of clinically resistant strains; evidence shows that β-lactamase of *M. catarrhalis* can protect other respiratory pathogens from β-lactam antibiotics when the pathogens are present in mixed infections (9).

It has been reported that a single dose of ampicillin is sufficient to prevent bacterial growth when the pathogen is sensitive, for example, in the case of *S. pneumoniae* or *H. influenzae* (10,11). Since sputum culture is still the cornerstone for diagnosis of LRTI, sampling prior to the administration of an antibiotic may affect the growth of a causative pathogen.

The increasing resistance of bacteria to antibiotics is being reported all over the world. β-lactamase production is the principal mechanism of antimicrobial resistance for both *M. catarrhalis* and *H. influenzae*. During the mid-1980s, 70-90% of *M. catarrhalis* strains were β-lactamase positive, and in several countries today, 100% of *M. catarrhalis* is β-lactamase productive (12-17). Gur et al. reported that 81% of the *M. catarrhalis* strains collected during 1996-1997 from a multicenter study in Turkey were β-lactamase producing (12). The present result of 100% β-lactamase producing strains reflects an increase in β-lactamase producers during this period.

France and Spain are also extensively affected by β-lactamase positive *H. influenzae* strains, while Germany, the Netherlands, and Italy show rates of approximately 16% (12-14, 16,17). In the Japanese pediatric population 4.3% of *H. influenzae* strains are β-lactamase positive (15). In Turkey, in a multicenter study, Gur et al. found that 7% of *H. influenzae* strains are β-lactamase positive (12), while a recent study showed that 6.1% of the *H. influenzae* isolated from patients with sinusitis is β-lactamase positive (18). We thus conclude that the resistance rate of β-lactamase is still not very high in the Turkish population.

Compared with other countries, penicillin-resistant *S. pneumoniae* is considerably low in Turkey (12,19). *S. pneumoniae* strains isolated between 1993 and 1999 from different parts of Turkey showed a variable rate (24-40%) of intermediate penicillin-resistant *S. pneumoniae* (20-23), which is consistent with the intermediate resistant strains found in the present study.

There is enormous concern and uncertainty regarding the treatment strategies that should be adopted to minimize increasing bacterial resistance. One school of thought – driven in part by concerns over the cost of therapy – advocates the use of older agents such as amoxicillin, in the hope that any resistance incurred will be to these agents, thus leaving the newer agents for select cases with acquired resistance (24). β-lactamase- producing *H. influenzae* and penicillin-resistant *S. pneumoniae* have remained at similar levels in Turkey for the past several years. Our personal experiences and the present study also reflect the fact that most physicians in Turkey prefer ampicillin for LRTI, and since there is no increase in antibiotic-resistant bacteria, we anticipate that this prescription practice will be continued.

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