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Dynamics of nasopharyngeal colonization by gram-negative rods in patients with resectable lung cancer during short-term hospitalization

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SUMMARY

We determined the frequency of colonization of nasopharynx by Gram-negative rods in 63 patients with lung cancer undergoing thoracic surgery who routinely receive antimicrobial prophylaxis. Throat and nasal specimens were taken from each patient twice: on the day of hospital admission (examination I) followed by thoracic surgery and on the fourth day after thoracic surgery (examination II). The isolated strains were identified using API 20E or API 20NE microtests. Susceptibility to selected antimicrobial agents was detected by the disc diffusion method according to Clinical Laboratory Standards Institute recommendations. A total of 27 strains of Gram-negative rods were cultured from 21 patients. During short-term hospitalization, in patients with lung cancer undergoing thoracic surgery and preoperative prophylaxis, qualitative and quantitative changes in Gram-negative rods colonizing the nasopharynx were observed. A statistically significant increase in the frequency of these bacteria on mucous membranes of nasopharynx in examination II was found (Chi-squared test, $p<0.00001$). The strains of *Enterobacteriaceae* were highly susceptible to antimicrobial agents, whereas most of the non-fermenting rods were classified as multi-drug resistant organisms.

KEY WORDS: Gram-negative rods, Upper respiratory tract, Antimicrobial agent susceptibility, Lung cancer

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INTRODUCTION

Respiratory tract infections, mainly bronchitis and pneumonia are an important medical problem in lung cancer patients. Such infections are due to colonization of the upper respiratory tract by potentially pathogenic and opportunistic microorganisms (Putinati *et al*. 1994; Duque *et al*., 1997; Rolston and Bodey, 1997; Zinner, 1999; Doddoli *et al*., 2001; Remiszewski *et al*., 2001; Berghmans *et al*., 2003; Sarihan *et al*., 2005). Immunocompromised lung cancer patients with dysfunction of airways defense mechanisms are particularly exposed to colonization by the hospital microflora, even during a short hospitalization lasting a few days. Exogenous sources of this microflora include food, water, hands of hospital personnel, sinks and hospital equipment such as respirators. A high percentage of cancer patients acquired *Pseudomonas aeruginosa* and *Klebsiella* species during hospitalization (Minah *et al*., 1986). It has to be stressed that during hospitalization, a patient's microflora and its susceptibility to antimicrobial agents is constantly changing depending on the environmental conditions and applied treatment, including the type of applied antibiotic therapy.

According to the literature, focused on the microbiological etiology of respiratory tract infections in patients with lung cancer, Gram-negative rods, belonging to *Enterobacteriaceae* family and

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non-fermentative rods are the major etiological factors (Minah *et al.*, 1986; Remiszewski *et al.*, 2001; Berghmans *et al.*, 2003; Czarniak *et al.*, 2003; Sarihan *et al.*, 2005).

The aim of this study was to assess the frequency of colonization of the upper respiratory tract by Gram-negative rods in patients with lung cancer, who routinely received antimicrobial prophylaxis before lung tissue resection. Antimicrobial susceptibility of the isolated strains was defined.

MATERIALS AND METHODS

Patient population

63 patients suffering from lung cancer aged 38-75 (57 men and 6 women, with a mean age of 61 years) were enrolled in the present study. Patients were qualified for the study after certain histopathological diagnosis. Histological type of lung cancer was determined before surgery on the grounds of examination of specimens obtained during bronchoscopy, thin needle biopsy, sputum cytology, supraclavicular lymph node biopsy and mediastinoscopy. Histological types of lung cancer of the studied patients are presented in Table 1.

In this group, non-small cell lung cancer was predominant, among which the most frequent was squamous cell carcinoma (29 patients; 46.03%), followed by large cell carcinoma (12 patients; 19.05%), adenocarcinoma (8 patients; 12.70%) and mixed cell cancers (7 patients; 11.11%). Seven (11.11%) patients had small cell lung cancer. During the preceding month all patients qualified for the study had not: suffered from airways infections, taken drugs influencing the immunological system, taken antimicrobial agents, had blood transfusion, suffered from allergic diseases. All patients were operated on in the Department of Thoracic Surgery of the Medical University in Lublin during 3 days after hospital admission. Each patient received preoperative antimicrobial prophylaxis according to hospital policy - piperacillin or cefuroxime; in some cases beta-lactam was in combination with amikacin. The patients did not receive any antimicrobial drugs after surgery. The study was approved by the Ethical Committee of the Medical University of Lublin. Informed consent was obtained from all patients.

TABLE 1 - *Histological type of lung cancer of studied patients.*

Histology	*No. (%) of patients (n=63)*
Squamous cell carcinoma	29 (46.03%)
Large cell carcinoma	12 (19.05%)
Adenocarcinoma	8 (12.70%)
Mixed	7 (11.11%)
Small cell carcinoma	7 (11.11%)

Microbiological assay

Throat and nasal specimens were taken twice: on the day of hospital admission (examination I) followed by thoracic surgery and on the fourth day after pulmonary resection (examination II). The swabs were immediately streaked onto appropriate nonselective medium (blood agar) and selective medium (McConkey agar). Plates were incubated for 24-48 h at 35°C under aerobic conditions.

The presence of Gram-negative rods in the upper airways (determined in throat or nasal swabs) in at least one sample was considered as colonization. The isolates were identified using Api 20E or Api 20NE and were tested by the disc diffusion method to the selected antimicrobial agents according to Clinical Laboratory Standards Institute (CLSI) recommendations.

Statistical analysis

Statistical analysis was performed by the Chi-squared test. Statistical significance was set at $p<0.05$.

RESULTS

A total of 252 throat and nasal specimens from 63 lung cancer patients were bacteriologically examined. Aerobic and facultatively anaerobic Gram-negative rods were found in 21 (33.3%) patients: in 5 (7.94%) patients on the day of hospital admission (examination I) and in 11 (17.47%) patients on the fourth day after thoracic surgery (examination II), while 5 (7.94%)

patients were colonized by bacteria of this group both in examinations I and II. A significantly higher frequency of Gram-negative rods in examination II was observed. The difference of nasopharyngeal colonization by these bacteria in successive examinations was statistically significant ($p<0.00001$).

The species of Gram-negative rods colonizing mucous membrane of upper respiratory tract in patients with lung cancer are shown in Table 2. A total of 30 isolates were cultured, including 19 (63.33%) and 8 (26.67%) belonging to *Enterobacteriaceae* family and non-fermentative rods, respectively; 3 (10.0%) isolates represented other gram-negative rods.

The incidence of nasopharyngeal colonization with non-fermentative rods was most frequently observed after thoracic surgery; only one isolate of this group was cultured in examination I. The most frequently isolated species were *Klebsiella pneumoniae* (4 isolates, 13.33%), *Escherichia coli* (4 isolates, 13.33%) and *Citrobacter freundii* (4 isolates, 13.33%); other commonly cultured gram-negative rods were *Acinetobacter baumannii* (3 isolates, 10.0%). Considering a localization of colonization, Gram-negative rods were more frequently isolated from throat (24 isolates, 80.0%) than nose (6 isolates, 20%, $p<0.00001$), including the strain of *Alcaligenes xyloxidans* cultured both from throat and nasal swabs obtained from one patient (Table 2).

The prevalence of Gram-negative rods on mucous membrane of nasopharynx in both examinations was found in 4 (6.35%) lung cancer patients. The following species were cultured in these patients: *Kluyvera* spp., *Escherichia coli*, *Citrobacter freundii*, *Serratia marcescens*. Moreover, in 1 (1.59%) patient in successive examinations rods belonging to different species (*Klebsiella oxytoca*, *Acinetobacter baumannii*) were found.

The mixed nasopharyngeal colonization with Gram-negative rods in 4 (6.35%) patients was identified: *Aeromonas hydrophila* + *Pasteurella aerogenes* in examination I and *Klebsiella pneumoniae* + *Escherichia coli*, *Acinetobacter baumannii* + *Ochrobactrum antrophi*, *Enterobacter*

TABLE 2 - *Gram-negative rods isolated from mucous membrane of the nasopharynx in lung cancer patients.*

Species	*No. (%) of isolates (n=30)*			
	T	*N*	*Ex. I*	*Ex. II*
Enterobacteriacae				
K. pneumoniae	4 (13.33)	-	1 (3.33)	3 (10.0)
E. coli	4 (13.33)	-	2 (6.67)	2 (6.67)
C. freundii	3 (10.0)	1 (3.33)	1 (3.33)	3 (10.0)
Kluyvera spp.	2 (6.67)	-	1 (3.33)	1 (3.33)
M. morganii	1 (3.33)	-	-	1 (3.33)
K. oxytoca	1 (3.33)	-	1 (3.33)	-
S. marcescens	-	2 (6.67)	1 (3.33)	1 (3.33)
S. odorifera	1 (3.33)	-	-	1 (3.33)
NFGNR				
A. baumannii	3 (10.0)	-	-	3 (10.0)
P. aeruginosa	-	1 (3.33)	1 (3.33)	-
A. xyloxidans	1 (3.33)	1 (3.33)	-	2 (6.67)
O. antrophi	1 (3.33)	-	-	1 (3.33)
A. radiobacter	1 (3.33)	-	-	1 (3.33)
Other				
A. hydrophila	1 (3.33)	-	1 (3.33)	-
P. aerogenes	-	1 (3.33)	1 (3.33)	-
Erwinia spp.	1 (3.33)	-	1 (3.33)	-
Total	24 (80.0)	6 (20.0)	11 (36.67)	19 (63.33)

NFGNR = nonfermentative Gram-negative rods; T = throat; N = nose; Ex. I = examination I; Ex. II = examination II.

TABLE 3 - *Patterns of mixed cultured Gram-negative rods isolated from mucous membrane of nasopharynx in lung cancer patients.*

No.	*Examination I*	*Examination II*
1	*Aeromonas hydrophila* *Pasteurella aerogenes*	-
2	-	*Klebsiella pneumoniae* *Escherichia coli*
3	-	*Acinetobacter baumannii* *Ochrobactrum antrophi*
4	-	*Enterobacter agglomerans* *Klebsiella pneumoniae*

TABLE 4 - *Patterns of antimicrobial agent resistance of Gram-negative rods isolated from mucous membrane of nasopharynx in lung cancer patients.*

Species	*Pattern of drug resistance*	*No. of isolates*
Enterobacteriacae		
K. pneumoniae	AM, SXT	1
K. pneumoniae	AM	2
K. oxytoca	AM	1
E. coli	AM, Te	1
C. freundii	SXT	1
Kluyvera spp.	AN	1
M. morganii	CIP	1
S. marcescens	AN, GM, Te, SXT	1
S. odorifera	SXT	1
NFGNR		
A. baumannii	PIP, TIC, CFP, CTX, ATM, AN, GM, Te, CIP, SXT, NN	1
A. baumannii	SAM, PIP, TZP, TIC, CFP, CTX, MEM, ATM	1
A. baumannii	AN, GM, NN	1
A. xyloxidans	Te	1
O. antrophi	CTX, ATM, AN, GM, Te, NN, CFP, CAZ, CTX, FEP, MEM, ATM, AN, GM	1
A. radiobacter	Te, CIP, SXT, NN	1

AM = ampicillin; AN = amikacin; ATM = aztreonam; CAZ = ceftazidime; CFP = cefoperazone; CIP = ciprofloxacin; CTX = cefotaxime; FEP = cefepime; GM = gentamicin; NN = tobramycin; MEM = meropenem; PIP = piperacillin; SAM = ampicillin/sulbactam; SXT = sulfametoxazole/trimethoprim; Te = tetracycline; TIC = ticarcillin; TIM = ticarcillin/clavulanic acid; TZP = piperacillin/tazobactam; Antimicrobial agents used for *Enterobacteriacae*: AM*; SAM*; PIP; CFP; CAZ = ceftazidime; CTX; FEP; MEM; ATM; AN;; Te; CIP; SXT; *not determined for *C. freundii, M. morganii, E. agglomerans, Serratia* spp.; Antimicrobial agents used for nonfermentative Gram-negative rods = NFGNR: SAM (not determined for *P. aeruginosa, A. xyloxidans, O. antrophi, A. radiobacter*); PIP; TZP; TIC; TIM; CFP; CAZ; CTX; FEP; MEM; ATM; AN; GM; Te**; CIP; SXT**; NN; **not determined for *P. aeruginosa.*

agglomerans + *Klebsiella pneumoniae* in examination II (Table 3).

The *Enterobacteriaceae* rods obtained in examinations I and II were characterized by high *in vitro* susceptibility to applied antibiotics and chemotherapeutics (Table 4). Five out of 17 strains belonging to this family showed ampicillin resistance, but all of them were sensitive to ampicillin/sulbactam combination. *S. marcescens* showed *in vitro* resistance to aminoglycosides, tetracycline and trimethoprim/sulfamethoxazole.

A much greater level of drug resistance was found in non-fermentative rods, over half of which were classified as multi-drug resistant strains (Table 4). Among the beta-lactam antibiotics used in the study, the most active *in vitro* against these rods was ticarcillin/clavulanic acid, followed by ceftazidime, cefepime and piperacillin/tazobactam. Among antibiotics and chemotherapeutics not belonging to beta-lactams the lowest resistance rate was observed with ciprofloxacine.

DISCUSSION

Lung cancer patients are at high risk for respiratory tract infections because intensive cancer therapy regimens as well as underlying disease cause both transient neutropenia and disruption of physical barriers in the airways. According to the literature (Berghmans *et al*., 1997; Hirakata *et al*., 1997; Remiszewski *et al*., 2001; Berghmans *et al*., 2003; Czarniak *et al*., 2003), frequent pathogens of nosocomial respiratory infections in this population of patients are Gram-negative rods because of their persistence in the hospital environment and broad antimicrobial resistance patterns (Esposito *et al*., 2007; Rossolini *et al*. 2007). *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* have been found in up to 68% patients with pulmonary infections.

Our preliminary observations indicate that Gram-negative rods dominated among bacterial species isolated from pleural drains fluid in lung cancer patients who were treated with surgery and received antimicrobial prophylaxis (Korona-Glowniak *et al*., 2003). A special emphasis was directed towards the trend of the changing distribution of these pathogens in the upper respira-

tory tract in the present study. It is worth noting that antibiotic prophylaxis was used routinely in patients who underwent pulmonary resection to prevent perioperative infections. However, it can also lead to development of antibiotic-resistant bacteria including opportunistic pathogens or other normally noninvasive microbial species, e.g. microorganisms belonging to natural microflora of human beings (Shay and Freifeld, 1999; Monroe and Polk, 2000; Klastersky and Aoun, 2004). Therefore, in our study antibiotic resistance patterns of isolated Gram-negative rods were analyzed.

Our data indicate that a "chosen" group of individuals - oncology patients suffering from lung cancer, undergoing thoracic surgery and receiving antimicrobial prophylaxis - acquired Gram-negative rods on nasopharyngeal mucous membranes even during short-term hospitalization. This is in accordance with the literature (Drakulovic *et al.*, 2001) that hospital microflora may colonize patients at the first week of hospitalization and this colonization may increase the severity of underlying disease and the process of healing after surgery.

Moreover, in our study in this short-term period of hospitalization qualitative and quantitative changes were observed in Gram-negative rods colonizing the nasopharynx. In the samples taken on the fourth day after surgery an increased prevalence of non-fermentative rods was observed. Most of these bacteria were multi-drug resistant. Similar trends of bacterial resistance have been documented by other authors (Jones, 2001; Van Loon *et al.*, 2005). In addition, the tendency to nasopharynx colonization with more than one species of Gram-negative rods after pulmonary resection was disclosed in our study.

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