Original Paper

Bacteorological Study of the Expectorant Except Koch Bacillus in Patients Interned in a Department of Pneumology

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ABSTRACT The study was conducted over a period of seven years in respiratory medicine department of the Municipal Hospital Caracal, which worked with 45 beds. During this period, 4862 patients were investigated. Bacteriological examination of sputum can be helpful in diagnosing bronchopulmonary infections if measure of sampling, decontamination and rapid processing are met. Bacteriological investigation of cough request must be made only when indicated, requiring a permanent and effective communication between clinician and laboratory doctor. Bacteriological investigation of cough request must be made only when indicated, requiring a permanent and effective communication between clinician and laboratory doctor. The pathological products for cyto-bacteriological examination must be accompanied by receipts, stating information on diagnosis, treatment with antibiotics on the way or completed, for the lab doctor to be endorsed on the arguments of pathology. Identifying microbial spectrum and the sensitivity of germs is the best means of argument for antibiotic choice; with empirical treatment there are significant differences from one region to another. Mycological examination is particularly important in diagnosing and detecting sputum superinfections and tracking the etiologic agent in some diseases rebellious to treatment with antibiotics.

KEY WORDS *sputum, bacteriological examination, pathogens germs, mycological examination*

Introduction

Harvesting and processing the material for bacteorological study

Sputum is composed of material from the lungs and tracheo tree as a result of disease process, typically mixed with saliva, which he joined in passing through the mouth.

So, sputum is a material removed from the presumed pathological source, often containing etiologic factors of disease, germs or parasites, and the reaction products of such organismuluicum pus, mucus, cells and serum protein of specific and nonspecific reaction.

The bacteorological exam of other germs except koch bacillus

Bacteorological exam technique

For the bacteriological examination itself, purulent portions are drawn by chance, which are submitted in a sterile Petri plate and wash with 2-3 ml of saline.
Sputum is likely to be very non-homogeneous in appearance and viscous, is absolutely necessary and important one fluidization, which simultaneously ensures a homogenous and possibly even a concentration of germs carried by subsequent centrifugation.

The examination itself

The microscopic examination. From the product taken and possibly liquefied, four smears are taken: methylene blue smear for a general assessment of the elements; a smear stained with Ziehl-Neelsen for acid-alcohol resistant bacillus; a smear Gram stained for gram-positive and gram-negative flora assessment; a smear stained May-Grunwald-Giemsa for cytology.

Smear examination consists of assessment of germs frequency, of morphological characters, of Gram and of cellular elements ratio.

Consider only the germs present between filaments of mucus and / or fibrin in polynuclear leukocytes and lymphocyte or near them. Give less importance to germs that "jam" large polygonal cells, originating from buccal epithelium and found in saliva. In the interpretation of microscopic examination, some information may be provided by cytological appearance, representing cells of the respiratory tree and blood cells. Thus these large flat homogeneous epithelial cells with relatively small nucleus are found in pharyngitis, nasopharyngitis epitelialei cells.

In bronchitis, asthma can be seen elongated cylindrical epithelial cells (bronchial origin) and the large alveolar cell lung congestion basophilia protoplasm. Also in pulmonary abscesses,

bronsiectazis, tuberculosis large cells with fat drops are characteristic.

Of blood elements, the most frequently encountered and most significant are polynuclear leukocytes, whose presence in relation to their frequency suggests a simple catarrh inflammation, inflammatory process, suppuration: the more it shows a degenerative aspect, the more it tends to suppurations greater. Lymphocytes can be seen in whooping cough and eosinophils characterize allergic catares (asthma). The presence of red cells suggests a process of sanguine extravasation.

Cultures. Proceed to seed the washed purulent portions or the material resulted by homogenization and fluidization on following materials:

- M.H. agar with blood;
- chocolat-agar;
- lactose-agar (AABTL).

These environments are seeded in duplicate for aerobic and carbon dioxide incubation. Depending on the outcome citobacteriologic examination and clinical data can add extra-blood agar Martin for anaerobic cultures, Sabouraud agar for yeast and a tube of broth thioglycollic, with subsequent subcultures. After 20 hours incubation at 37 degrees, examine the plate, noting the frequency of cultures, noting the cultures frequency and the appearance of colonies on each medium and making a quantitative assessment between the development of germs exclusively developed on blood agar and observing the environment AABTL flora. For each type of colony, redrops are made on appropriate medium and identified according to specified procedures.

Interpretation of cultures results. This test rarely gives reliable results, though it requires time and repeated labor.

Very often the sputum during harvest gets contaminated by oro-pharyngial flora, so it is very difficult to say which of the potential pathogenic germs true etiological agent is. Thus, cultures with non-pathogenic neisserri, white staphylococcus, viridans streptococcus, the pseudodifterics are almost certainly the result of contaminated sputum with oro-pharyngial flora.

Instead, a good correspondence between the examination of citobacteriologic and cultures, especially for development of a culture almost monomorphic with most likely found in respiratory tree infections (streptococcus pneumoniae, Haemophilus influenzae, beta-hemolytic streptococcus, staphylococcus, Klebsiella), with the existence of a corresponding cytological support (epithelial cells of bronchial or alveolar origin, polynuclear leukocytes in whole or degraded), constitute elements of a true result.

Besides the above mentioned germs, in infections in the respiratory tree - except for Mycobacterium tuberculosis - can be found constant pathogenic germs (Bordetella pertussis, Bacillus anthracis, Corynebacterium diphteriae toxigenic) and species pathogenic conditioned like: Acinetobacter group, E. coli , Proteus, Pseudomonas, Pasteurella group, Escherichia group).

In the event of a true positive result of the cultures it absolute necessary antibiogram to be made, if the isolated germs have a various sensitivity to antibiotics and chemotherapy.

As a final conclusion criteria for assessing the pathogenicity germs were: purulent sputum (over 50% inflammatory cells per smear); phagocytosis; relationship between the germs cultivated on medium and those observed on the smear; lack of growth on cultivated medium in aerobic and the presence of germs on smear (for anaerobic germs).

Results obtained for the bacteorological exam of the sputum

There were identified the following types of germs that fulfilled the pathogenic criteria (Table 1): Haemophillus influenzae 263, anaerobe germs 168, Klebsiella pneumoniae 61, Pseudomonas aeruginosa 53, Streptococ. pneumoniae 20.

Analyzing the patients admitted and studied in 647 cases (Table 1), depending on the discharge diagnosis the following are highlighted: infectious exacerbations of BPCO - 289 case, the breakthrough in the evolution of pulmonary tuberculosis - 98 cases, chronic suppurations -132 cases, bronsiectasis - 68 cases and lung abscess – 32 cases.

Table 1: Frequency of germ types identified in the sputum of patients

Types of germs	Nr. cases	%
Anaerobes	168	26
Anaerobes + H. influenzae	12	1,8
Anaerobes + Kl. pneumoniae	10	1,5
Anaerobes + Pseudomonas aeruginosa	6	0,9
H. influenzae	263	40,8
H. influenzae+ Strep. Pneumonie	12	1,8
H. influenzae + Staph. aureus	11	1,7
Kl. Pneumoniae	61	9,4
S. pneumoniae	20	3,1
Ps. Aeruginosa	53	8,2
Staph. aureus	31	4,8
TOTAL	647	100

Correlation between diagnosis obtained at discharge and type of germ isolation show a high frequency of Haemophillus influenzae disease in all studies (Table 2-3-4-5).

Table 2: Types of pathogens isolated from patients with bronsiectasis

Pathogens	Nr. cases	%
Anaerobes	18	26
H. influenzae	24	35,3
Kl. pneumoniae	15	22
Str. Pneumoniae	7	10,3
Ps. aeruginosa	3	4,4
Staph. Aureus	1	1,5
TOTAL	68	100

Table 3: Types of pathogens isolated from patients with BPCO

Pathogens	Nr. cases	%
Anaerobes	76	26,3
H. influenzae	143	49,5
Kl. pneumoniae	38	13,1
Str. Pneumoniae	21	7,3
Ps. aeruginosa	7	2,4
Staph. Aureus	4	1,4
TOTAL	289	100

Table 4: Types of pathogens isolated from patients with pulmonary abscess

Pathogens	Nr. cases	%
Str. Pneumoniae	6	18,8
Anaerobi	11	34,4
H. influenzae	13	40,6
Ps. aeruginosa	1	3,1
Staph. Aureus	1	3,1
TOTAL	32	100

Table 5: Types of pathogens isolated from patients with chronic suppuration

Pathogens	Nr. Cases	%
Anaerobes	39	29,5
H. influenzae	51	38,7
Kl. Pneumoniae	22	16,7
Ps. Aeruginosa	14	10,6
Str. Pneumoniae	6	4,5
TOTAL	132	100

In patients studied were made antibiogrammes which should the germs sensibility to antibiotics that could be hypothetical used in treatment of the studied diseases (Table 6, 7, 8, 9 and 10).

Table 6: Sensibility to antibiotics for Staph. aureus

Antibiotic	Nr. cases with resistence	% cases with resistance
0xacilin	9	29
Erythromycin	11	35,5
Clindamycin	4	12,9
Ampicillin sulbactam	23	74,2
Amoxi+ac. Clavulanic	25	80,6
Cephtazidim	8	25,8
Cephalexin	3	9,7
Biseptol	16	51,6
Ciprofloxacin	10	32,3
TOTAL	31	100

Table 7: Sensibility to antibiotics for Pseudomonas aeruginosa

Antibiotic	Nr. Cases with resistance	%
Colistin (polymyxina)	3	5,6
Gentamicin	6	11,3
Nalidixic acid	46	86,8
Ciprofloxacin	4	7,5
Amoxicillin	51	96,2
Tetracycline	33	62,3
Total	53	100

Table 8: Sensibility to antibiotics for Klebsiella pneumoniae

Antibiotic	Nr. Cases with resistance	%
gentamicin	13	21,3
ofloxacin	5	8,1
cefataxim	12	19,6
biseptol	22	36,1
tetracycline	31	50,8
Nalidixic acid	15	24,6
Total	61	100

Table 9: Sensibility to antibiotics for Haemophilus influenzae

Antibiotic	Nr. Cases with resistance	%
Amoxi+ ac clavulanic	42	16
Ampicillin sulbactam	55	20,9
gentamicin	47	17,9
biseptol	63	24
tetracycline	77	29,3
cloramphenicol	89	33,8
Total	263	100

Table 10: Sensibility to antibiotics for anaerobes

Antibiotic	Nr. Cases with resistance	%
Amoxi+ ac clavulanic	41	20,8
Ampicillin sulbactam	35	24,4
cloramphenicol	44	26,2
clindamycin	62	36,9
nalidixic acid	132	78,6
colistin	143	85,1
Total	168	100

Discutions

Cytobacteriologic examination of sputum is a method which is commonly used but if not executed after a proper methodology can give erroneous results.

The presences in the oropharyngeal cavity of some species of potentially pathogenic germs that may contaminate secondary bronchial secretions constitute a major impediment to identify the germ responsible for disease process.

Even if conditions were properly met for contamination mitigation, a too long a time elapsed from harvest to processing time may compromise the outcome of allowing abundant growth of flora in this case were contaminated at a low percentage.

The presence of Enterobacteriaceae in sputum may be due to contamination emitted spontaneously, whereas for patients with a serious condition these germs populate the oropharyngeal abundantly.

Pathogen identification requires a large number of laboratory techniques, being imposed by diversity germs potentially involved. (Kuchmeister 1973)

The germ which was mainly in patients being studied was Haemophillus influenzae over 40.8%, followed at a large distance by the presence of

anaerobes in 26% and then Klebsiella pneumoniae in 9.4% and Pseudomonas aeruginosa in 8.2%, data were superimposable over the ones in medical literature. This data is comparable to those in the study of Canton, Valderice (1977).

In this study, data can be compared with those in literature, could see also the predominant Haemophillus influenzae (49.5%) in sputum of patients with acute COPD, followed by anaerobes (26.3%), Klebsiella (13.1 %) and S. pneumonae at a rate of only 7.3. (Bercea Dinulescu 1983; Bals, Diaconescu 1983; Boganescu 1980).

Bronsientasis are another diagnose entity being investigated through the bacteriologic exam of the sputum. Generally Haemophillus influenzae isolates (35.3%) and anaerobic (26.5) and, for those with a history of multiple treatments with antibiotics, can be isolated bacteria species as Staphilococus aureus and Pseudomonas aeruginosa as are cited in literature.

The data found is superimposable with those of literature studied (Pujans, Gallego, 1977) as a rate of 41.2% was found for Haemophillus influenzae in sputum of patients ultimately diagnosed with bronsiectasis. Also, was found an increased frequency of appearance in spit of Klebsiella (17.6%) and 19.5% anaerobes probably in cases of multiple treatments with antibiotics administered to these patients.

For the studied patients, with lung abscess that occurred after a pneumonia that has secondarily excavated itself with loss of lung tissue, were isolated predominantly Haemophillus influenzae (40.6%) and anaerobic (34.4%). Streptococcus pneumonia followed with 18.8%, a percentage much lower than the one in a real population, this being explained by the fact that usually pneumonia with typical evolution without serious forms arrive and are treated in departments of Internal Medicine and Infectious Diseases. (Diaconescu, R. Tucra 2001)

Chronic suppurations treated for several years and pulmonary abscesses are mainly caused by anaerobic pathogens, the major pathogens involved in these diseases being those of the groups Fusobacterium, Peptostreptococus (sensitive to penicillin and cefasporine) and Bacteroides (resistant to penicillin). These data are similar data in medical literature by the works studied, Bercea A (1978), Diaconescu (2001).

Also was found that many bacteriologic examinations of sputum were performed on patients eventually diagnosed with lung cancer or pulmonary tuberculosis. This could be explained by the presence of large lesions occurred secondary (necrosis, bronsiectasis) which can overgrowth evolving as a suppuration as also shown in Erozan Y., J. Frost, K (1970).

After determining the type of pathogen causing the infection, sometimes was necessary to establish its sensitivity to antibiotics that could be used to treat the patient. Some laboratories studied the sensitivity of germs in certain areas over a period of time. Results are normally used as an indicator for choice of antibiotic in the appropriate therapy.

It was determined the sensitivity to antibiotics, especially S. aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and anaerobes.

For S. aureus the lowest chimioresistance was observed for Cefalexim, a cephalosporin 9.7%, then Oxacilin 29% and the highest for clavunic amoxi+acid in a percentage of 80.6%. Different penicillins were studied,as Clindamicin and modern mixes of clavulanic amoxicillin+acid (Generen 1988) and sulbactam ampicillin, that are frequently used especially for outpatient treatment.

Beta-lactamases are enzymes produced by pathogenic bacteria, which encircle and rapidly inactivate the antibiotic molecules before they have time to act. These enzymes are then regenerated and become active against other antibiotic molecules.

Beta-lactamases are responsible for 80% of bacterial resistance to beta-lactams antibiotics (penicillins and cephalosporins). Many pathogens, often involved in nosocomial infections are producer of beta-lactamases such as in our study Staphilcoccul aureu.

Sulbactam is a penicillin-class molecule that has a high affinity for beta-lactamases. When "attacked" it is fractured and irreversibly binds to the enzyme preventing their regeneration. (Generen 1988; Hampel, Lode, Brucker 1998)

Used in combination, the sulbactam protects the ampicillin from the action of beta-lactamases, allowing it to reach the bacterial cell receptors. Reaching this level, the antibiotic inhibits cell wall formation, causing membrane rupture and bacteria destruction (Hampel, Lode, Brucker, 1998). This resistance given to antibiotics with ampicillin sulbactam was caused because previously unprotected ampicillin was used which resulted a resistance which ell overlapped modern drugs.

With regard to Pseudomonas aeruginosa antibiogram, least resistance was observed in Colistin (polymyxin) with 5.6%, Norfloxacin 7.5%, followed by Gentamicin from glycosides family in percentage 11.3%. This germ was observed to have a very balanced chimioresistence

to antibiotics when studied "in vitro". The same data were communicated by NEU in 1984.

In Klebsiella pneumoniae it was observed a chimiosensibility to studied antibiotics, highest being for ofloxacin with a resistance of only 8.1%.

Haemophillus influenzae has presented a chimiosensibility to most of the antibiotics used in this study, most sensitive antiobiotic being clavulanic amoxicillin+acid with a resistance of 16%.

Regarding the anaerobes, only the chimioresistance "in vitro" of sulbactum ampicillin (24.4%) has been taken in discussion (Nicholas 1985).

The data obtained correspond with small exceptions with that of literature. The vast majority of germs tested showed multiple resistances to antibiotics, because many of the patients investigated were not at their first treatment with antibiotics and were dragged. Prior treatment in most cases was defective leading to secondary chimioresistance to the applied antibiotic.

Mycological examination of sputum

Mycological examination was conducted in all products parallel to the bacteriologic examination, consisting of bacterioscopic examination of the products and seeding in Sabouraud medium.

Direct exam

For the microscopic examination, the material collected will be deposited on a well degreased glass slide, upon which it was put 1-2 drops of the used dissociating solution, everything being covered by a thin glass slide. The excess of substance which exceeds the margins of the glass slide will be absorbed with filter paper.

For the microscopic examination, it can be obtained: ram yeast cells, with or without pseudomicellis (in oral candidiadis); micelien filaments segmented in rectangular cells of 4-8 microns, with edges more or less rounded, are found in Geotrichum; long, branched filaments accompanied by lots of small (2-3 microns), round, grey-green colored spores, which do not bud, are found in sputum which contains Aspergillus; levuriform elements, looking round or oval cells with thick membrane, with double contour, surrounded by a mucilaginous capsule which is reproduced by unipolar budding found in sputum produced by Cryptococcus. (Kuchmeister 1974)

All items found in cough have diagnostic value only if their presence is very large being conclusive especially if they are found in sputum harvested by bronchoscopy. In other cases (except Cryptoccoccus), their chance appearance is not taken into account, being considered ordinary saprophyte of mucous membranes. (Paun, Anastasatu 1983)

Examination through culture

For specifying the specie of the isolated fungal agent of the pathologic product is required sowing made on culture media, being used for isolation of the dermatophytes and fungi mushrooms the Sabouraud medium.

For the successful sowing of the pathogenic material on the culture mediums will be taken into account the following recommendations:

- the ansa is buckled, cooled down in the condensing liquid from the medium tube, afterwards the material is lifted and deposited on the surface of the culture medium, in different points, 3-4 fragments per tube.
- the seeding must not take more than 40-50 seconds.
- the seeding must take place away from draft (open windows, ventilation) and in a sterile atmosphere (special box, using uv lamps)
- it is recommended the seeding of more tubes 2-3.
- in profound microzoa.
- the grains are first washed in a sterile Petri box half an hour with sterile saline or distilled water, to which is added (especially for black grains) a large action spectrum antibiotic, to remove possible bacterial flora.
- grain's seeding is done with the ansa, 2-3 grains per culture medium, larger grains can be fragmented.
- it is recommended a large number of tubes to be seeded, up to 15-20, some grains being sterile and not able to evolve the culture.

For diagnosis and determining the developed species, will be taken into account the following criteria:

- the necessary time for developing the cultures varies from one specie to the other.
- the macroscopic aspect of the colony can be:
- mat, glossy, fluffy or dusty surface;
- raised, lowered, radiated or cerebifom relief (central)
- the color of the surface of the colony and its reverse.
- the possible presence of a pigment that spreads in the medium.
- the consistence of the colony: cracky, creamy, pasty.
- the microscopic aspect is determined by harvesting of a fragment from the examined colony, which is deposited on a glass slide, over

which 2-3 drops of potassium hydroxide or other dissociant is poured and is examined through the microscope. The ansa with which the harvesting is made must me sterilized through buckling. The harvesting will be made at surface, as well as in depth of the colony.

From Table 11, it can be observed that positive mycological examination was high for the diseases: pulmonary tuberculosis 313 cases, obstructive chronic bronhopneumopaty with or without chronic pulmonary heart (104) and pulmonary abscesses and suppurations (84). This data was extrapolated at a total of 646 cases found positive at the mycological examination. This data corresponds with the one announced by Leophonte in 1994.

Table 11: Mycological examination and the type of disease studied

Disease	Nr.	%
TBC	313	48,5
Bronho-pulmonary suppurations	84	13
BPCO+CPC	104	16,1
Pleurisy	18	2,8
Bronho-pulmonary neoplasm	118	18,2
Other diseases	9	1,4
TOTAL	646	100

In Table 12, it can be observed instead that the positive mycological examination was present in patients dragged or with frequent antibiotics treatments in their history. The percentage obtained this way is part of the entire patients studied. Lung neoplasms was 28.4% of cases with secondary mycosis being the most frequent, if overlapped with an inflammatory lung disease that percentage became almost l00. Afterwards were the ailments; lung abscesses and suppurations for 31.3%, then chronic broncho-pneumopatis 16% and pulmonary tuberculosis 13.8% (in the latter case more frequent were the cases of chronically ill, the Therapeutic failure, failures and readmission). (Leophonte 1994, Munteanu Strambu 1998).

Table 12: The positive mycological exam in sputum examination

	NR	%
TBC	313	13,8
Bronho-pulmonary suppurations	84	21,2
Bpco+ cpc	104	16
Pleurisy	18	8,5
Bronho-pulmonary neoplasm	118	28.4
Other	9	11,4

In Table 13, the obtained mycological exam was divided in four groups:

Group I filaments on smear with negative culture represent a contamination of sputum at passing through the oral cavity, representing the highest percentage of 76 and 491 cases respectively.

Group II, yeast cells ram, pseudomicelis on smear and positive culture represents the existence of a pulmonary candidiasis in these patients, present in this study for 10.4% of the patients studied.

Group III represents patients with positive cultures with few colonies, and the smear is negative for candidiasis (superinfection), present in 36 patients (6.6%).

Group IV, filaments (pseudomicelii) and positive culture which is also massive which also represent the existence of a pulmonary candidiasis, no adequate medical treatment existing for 52 patients.

Table 13: Results of the mycological exam

Mycological exam	Nr.	%
Group I	491	76
Group II	67	10,4
Group III	36	6,6
	52	
Group IV		8
TOTAL	646	100

From this table it emerges that contamination of oral flora was large, thus the results of the mycological examination being distorted.

Smears that were used in this work were performed in the clinical laboratory Caracal County Hospital and worked in the microbiology department at the University of Medicine Craiova at the pathology service.

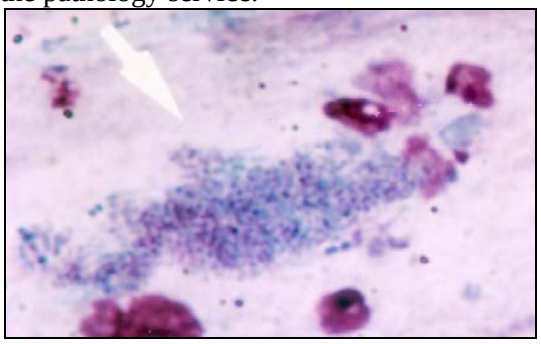

Photo 1 Polymorphic flora M.G.G. stain

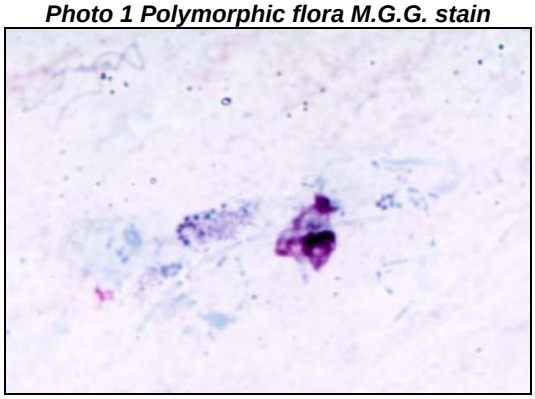

Photo 2 Polymorphic flora (neutrophil and bacillus)M.G.G. stain

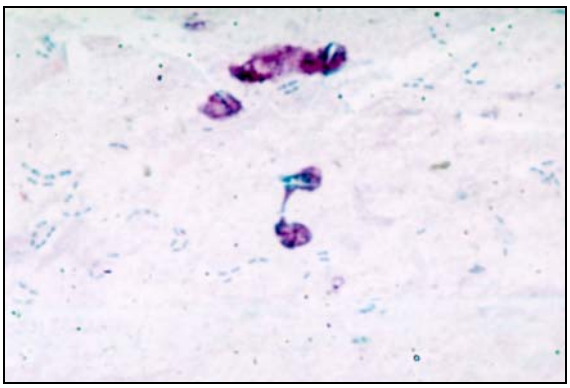

Photo 3 Microbial flora (cocci in encapsulated diplo) M.G.G. stain

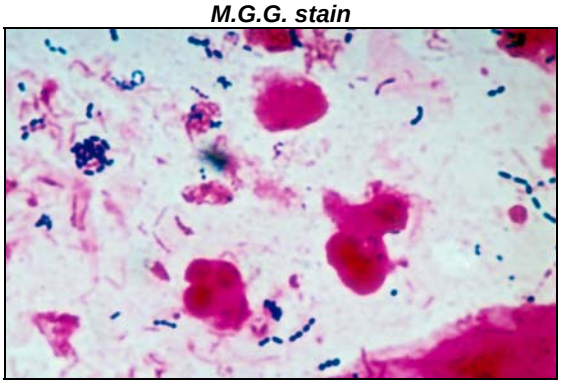

Photo 4 Positive Gram Cocci in diplo, neutrophil chain Gram stain

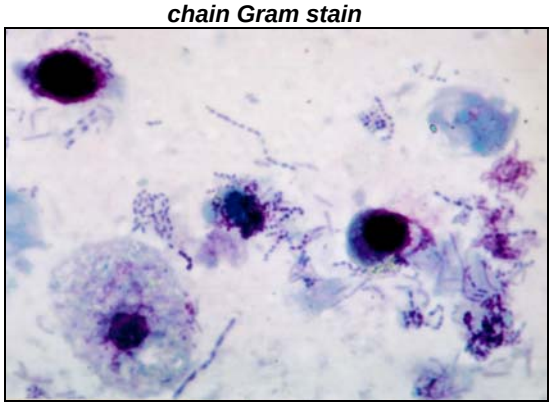

Photo 5 Macrophages with inclusion and flora M.G.G. stain

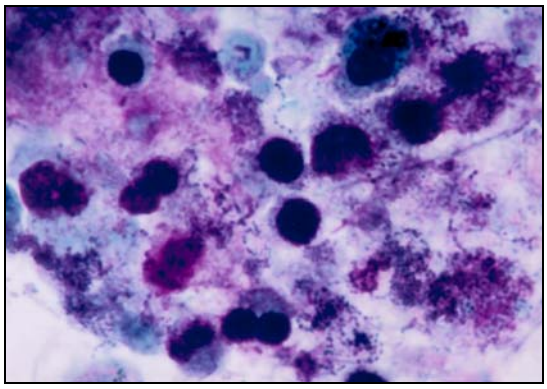

Photo 6 Neutrophil and flora M.G.G. stain

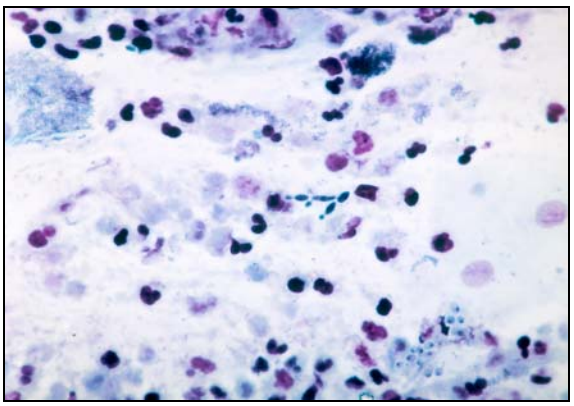

Photo 7 Yeast cells of candidiasis and neutrophil M.G.G. stain

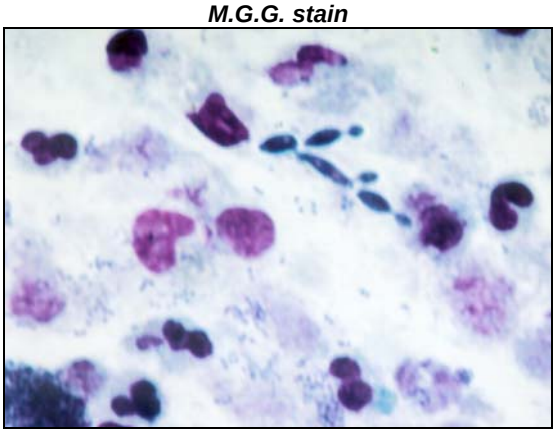

Photo 8 Detail M.G.G. stain

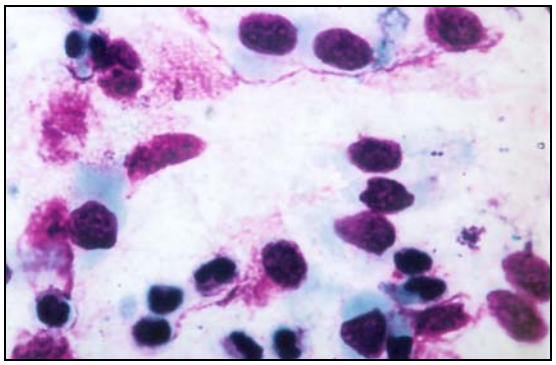

Photo 9 Cylindrical cells, neutophils and flora M.G.G. stain

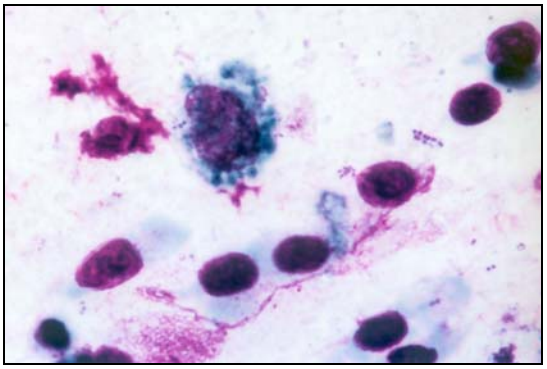

Photo 10 Macrophages, cylindrical cells and flora M.G.G. stain

Conclusions

1. Bacteriological examination of sputum can be helpful in diagnosing bronchopulmonary infections if measure of sampling, decontamination and rapid processing are met.

2. Bacteriological investigation of cough request must be made only when indicated, requiring a permanent and effective communication between clinician and laboratory doctor.

3. Bacteriological investigation with germ identification and antibiogram is a laborious method required in special situations (serious infection, dragged person, treatment failure, nosocomial infections).

4. The pathological products for cyto-bacteriological examination must be accompanied by receipts, stating information on diagnosis, treatment with antibiotics on the way or completed, for the lab doctor to be endorsed on the arguments of pathology.

5. Identifying microbial spectrum and the sensitivity of germs is the best means of argument for antibiotic choice; with empirical treatment there are significant differences from one region to another.

6. Mycological examination is particularly important in diagnosing and detecting sputum superinfections and tracking the etiologic agent in some diseases rebellious to treatment with antibiotics.

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