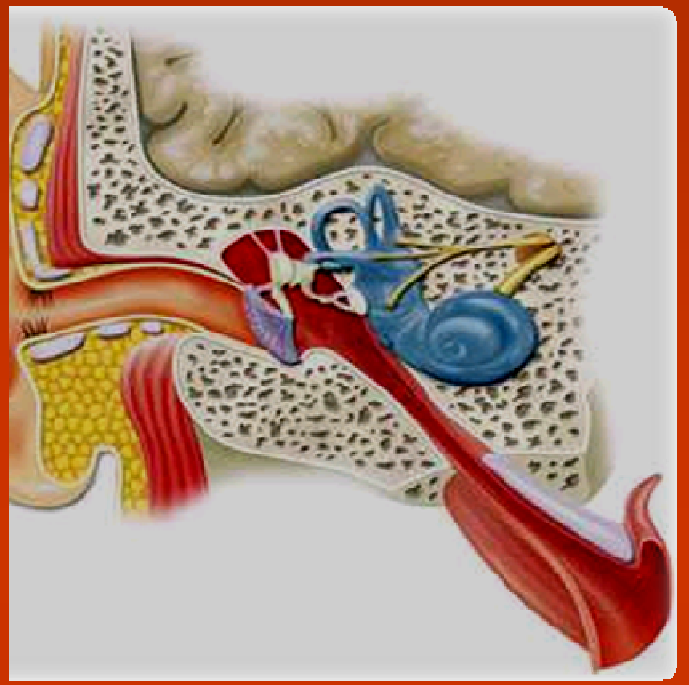


Specific microbiota isolated from cats' ears from Western Romania

www.veterinarypharmacon.com

Materials and methods

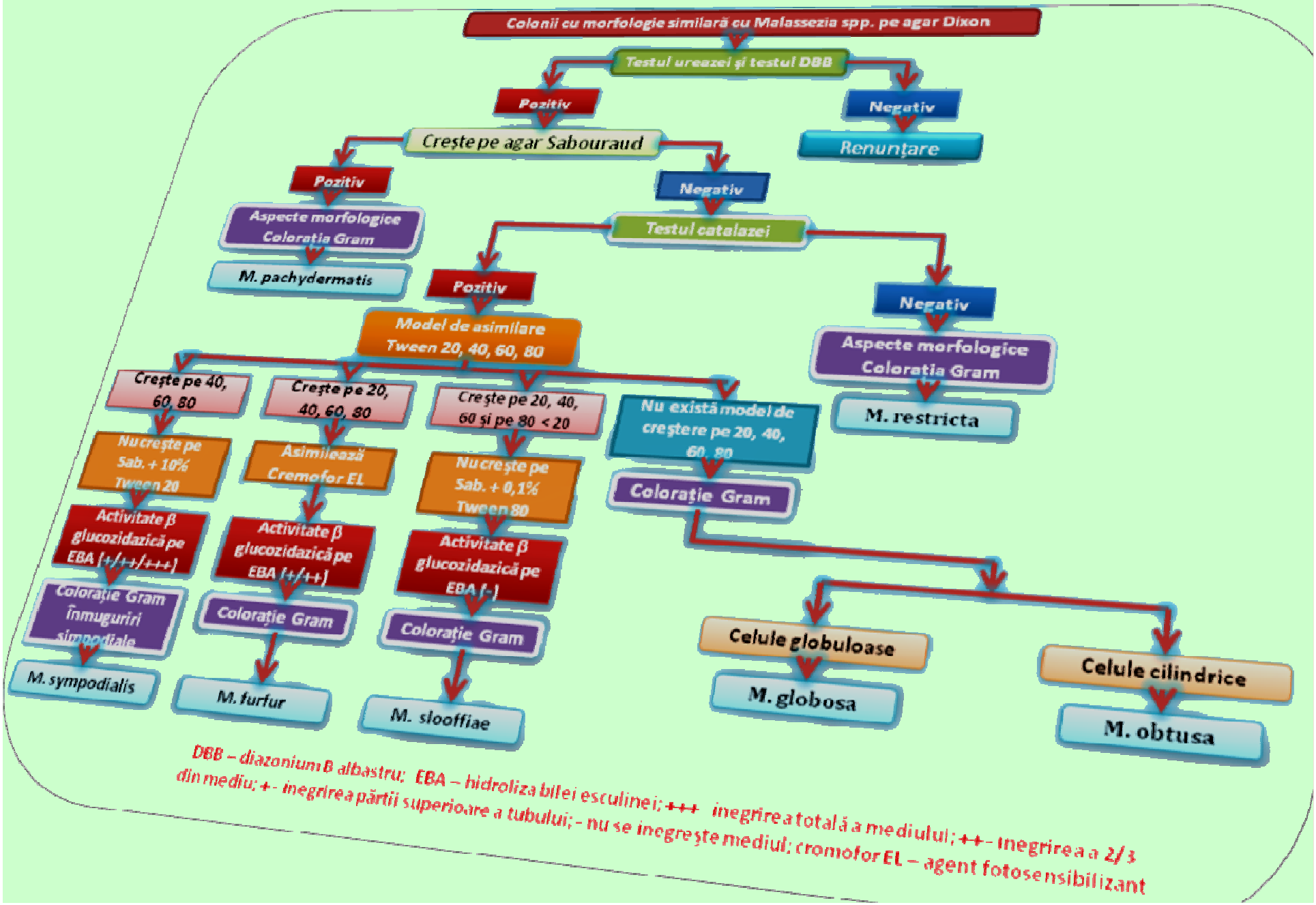
Animals

- 42 house pet cats (19 males and 23 females)

Methods

- Samples: - collected using swabs,
- Cultures: - Sabouraud Glucose Agar (SGA),
  - SGA supplemented with olive oil (10ml/litre), and
  - Leeming's medium
  - Media contained:
    0.05% chloramphenicol + 0.05% gentamicyne

Stages of isolation and identification of *Malassezia spp.*

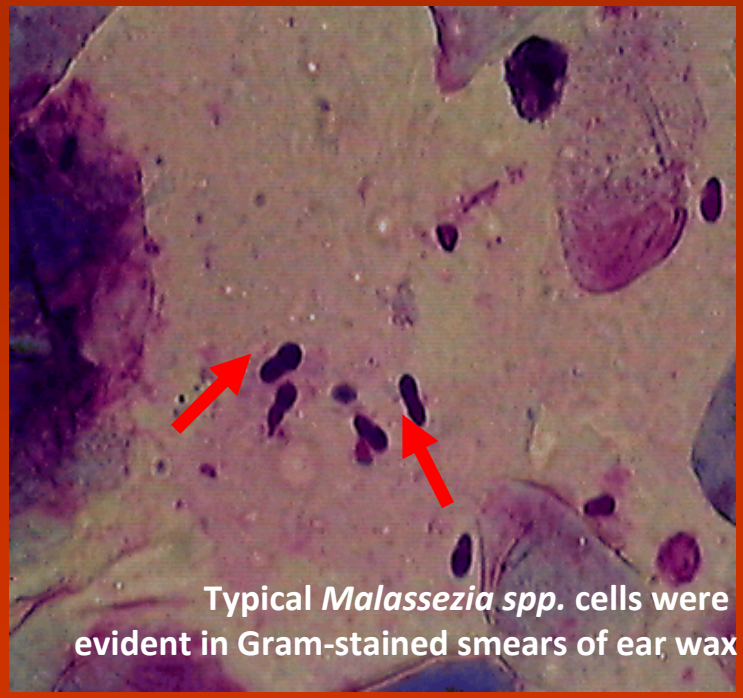


DBB – diazoniu B albastru; EBA – hidroliza bilei esculinei; +++ înegrirea totală a mediului; ++ - înegrirea a 2/3 din mediu; + - înegrirea părţii superioare a tubului; - nu se înegreşte mediul; cromofor EL – agent fotosensibilizant

- Each ear wax smear was heat fixed and Gram stained

Microscopy:

- To identify: *Malassezia spp.* typical cells' presence
- Plates: incubated at 35°C, examined at 3, 5, 7, and 14 days.
- When growth was detected, five different colonies were selected from the SGA supplemented with olive oil and from the Leeming's medium and subcultured on SGA to determinate their lipidic dependence.

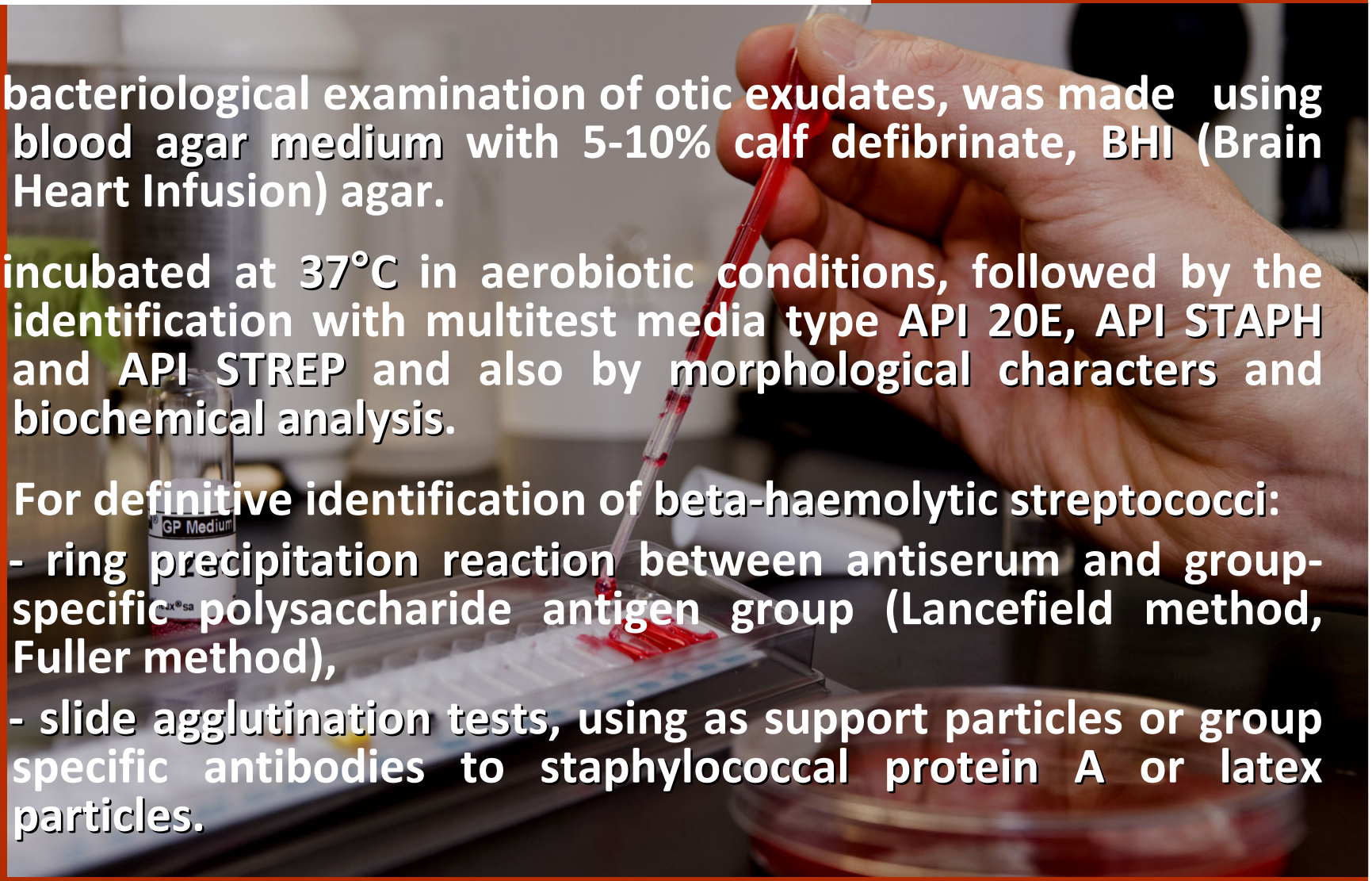


Typical *Malassezia spp.* cells were evident in Gram-stained smears of ear wax

Malassezia spp. cultural aspects: cream, smooth, and umbonated colonies

- Typical *Malassezia* cells were evident in the Gram-stained smears of ear wax from 38 cats.
- From 21 cats, *Malassezia,* proved to be positive and isolated in culture (29.57%).
- In 15 cats (21,12%), only *M. pachydermatis* was isolated during the first week of incubation.
- A lipid-dependent specia was isolated from one cat at 7 days of incubation. The isolate formed cream, smooth, and umbonated colonies

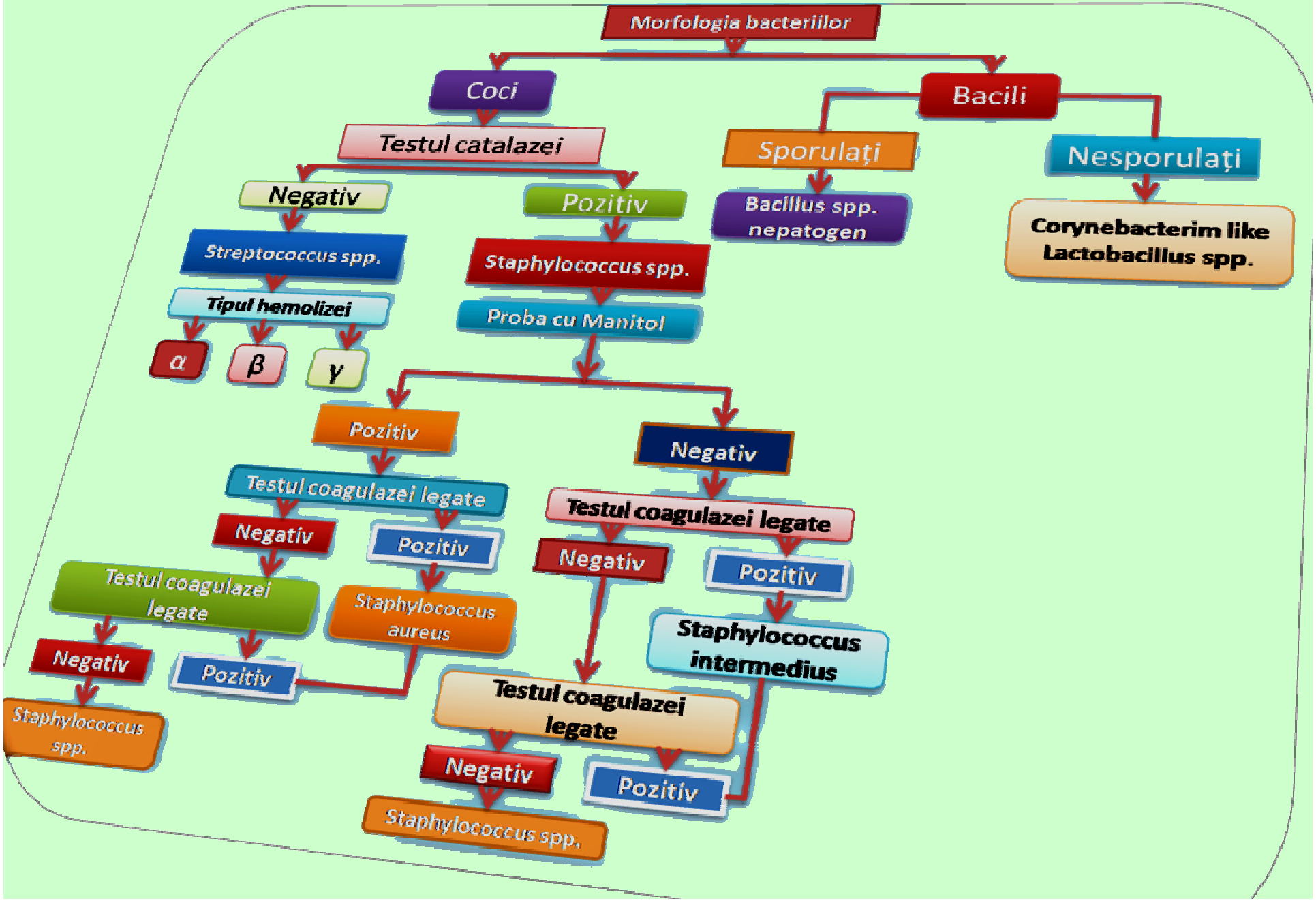
Microbiological examination



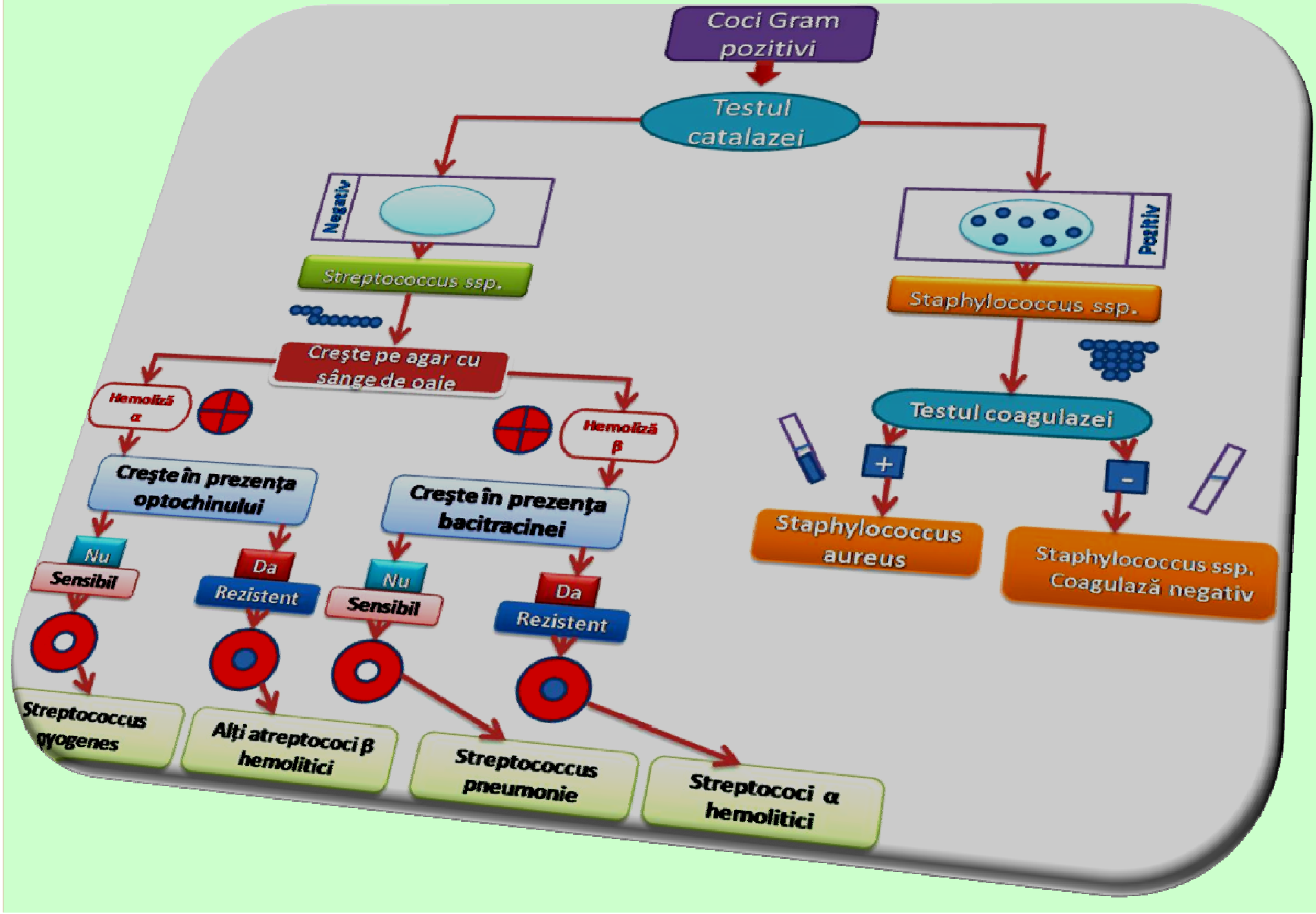
- bacteriological examination of otic exudates, was made using blood agar medium with 5-10% calf defibrinate, BHI (Brain Heart Infusion) agar.
- incubated at 37°C in aerobiotic conditions, followed by the identification with multitest media type API 20E, API STAPH and API STREP and also by morphological characters and biochemical analysis.
- For definitive identification of beta-haemolytic streptococci:

  - ring precipitation reaction between antiserum and group-specific polysaccharide antigen group (Lancefield method, Fuller method),
  - slide agglutination tests, using as support particles or group specific antibodies to staphylococcal protein A or latex particles.

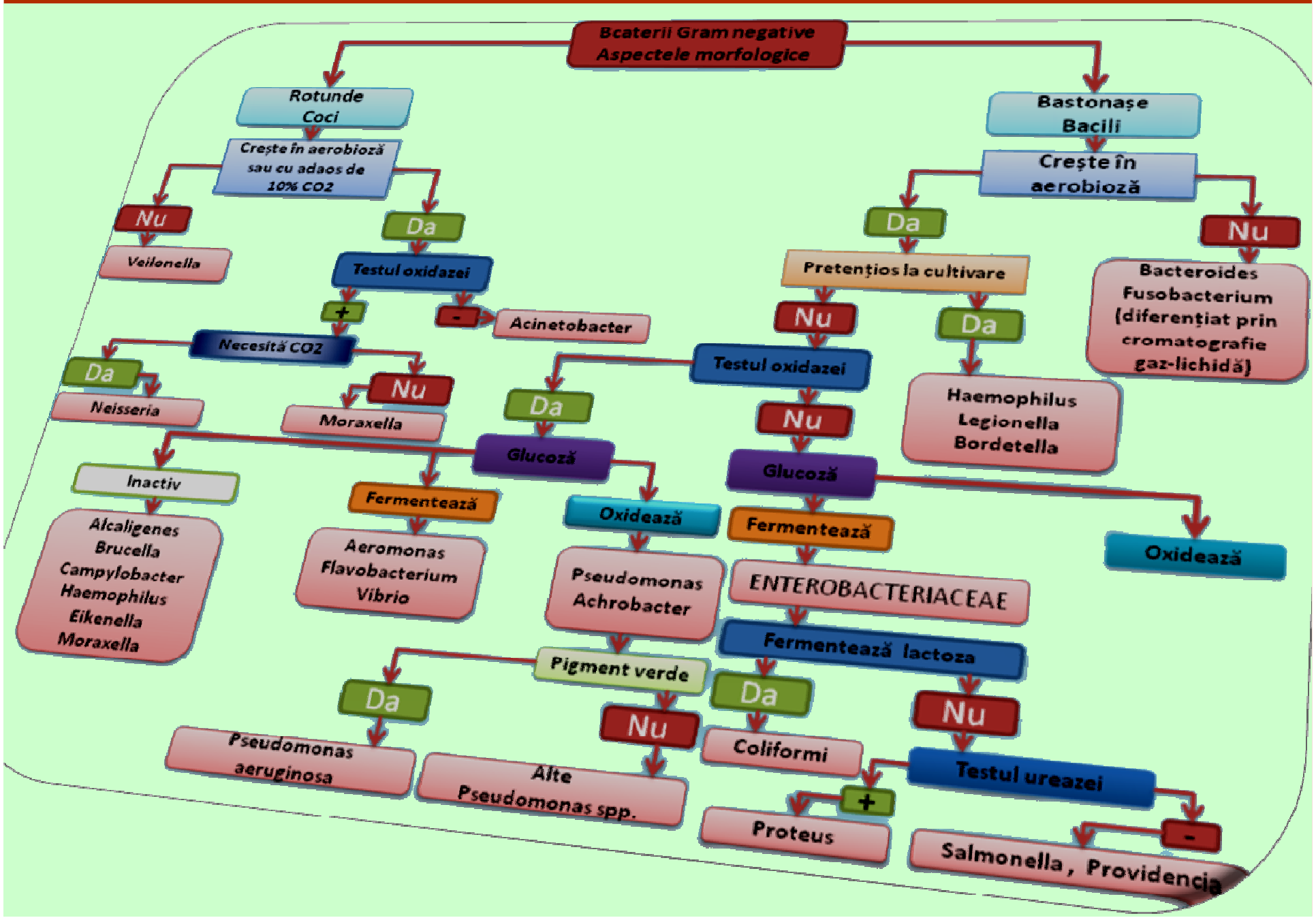
Isolation and identification of Gram pozitives



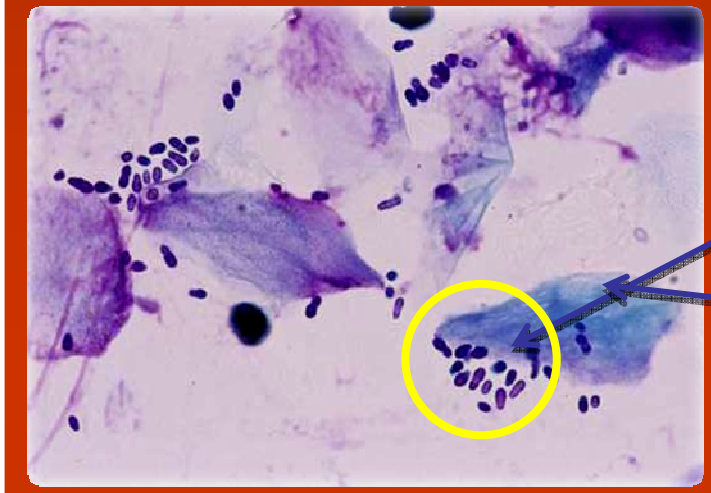
Isolation and identification of coci Gram pozitives



Isolation and identifiation of Gram negatives



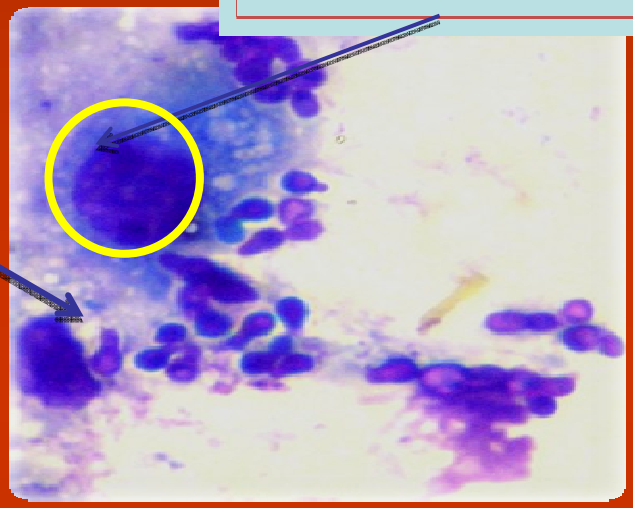
Citologic exam: Specific recognition elements

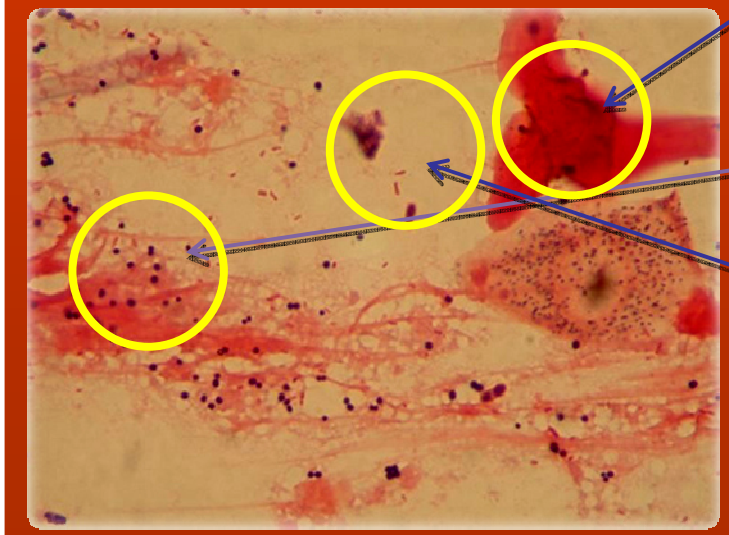


Malassezia

Epithelial anucleate cels

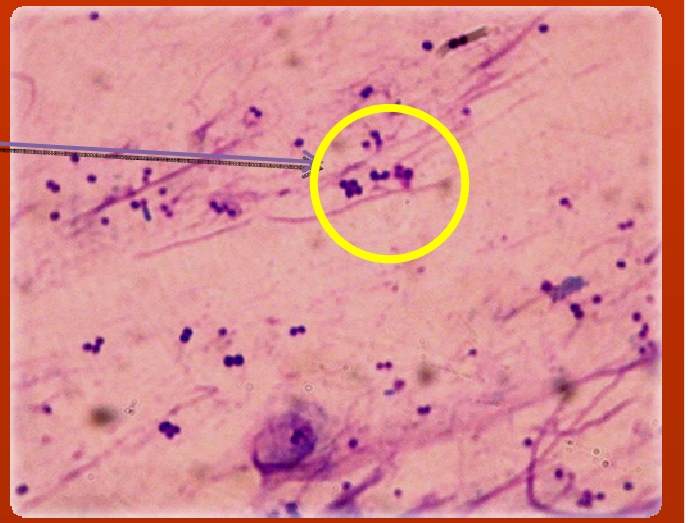
Epithelial nucleate cels





Coci Gram +

Bacili Gram -



Results

- 71 strains of microorganisms were isolated, of which:
  21 of *Malassezia* and 7 strains of *Microsporum canis.*
- Associations of bacterial strains with *Malassezia* was established

Microorganisms isolates from external auditory canal, from cats with otitis externa

Microorganism	Isolated strains	Total isolates %	Type of otitis
Malassezia spp.	21	29,57	-
▪Pure culture	2	9,52	EC
+ Streptococcus group G	1	4,76	S
+ Proteus ssp.	3	14,28	S
+ Pseudomonas aeruginosa	6	28,57	S
+ S. aureus	2	9,52	S
+ Pasteurella spp.	4	19,04	S
+ Staphylococcus spp.	3	14,28	EC
Microsporum canis	7	9,85	-
▪Pure culture	7	100	EC

Note:
EC - Acute erythematous-ceruminous
S – chronic - suppurative

Results of bacteriological and mycological examination, in fellowship with clinical otitis to cats

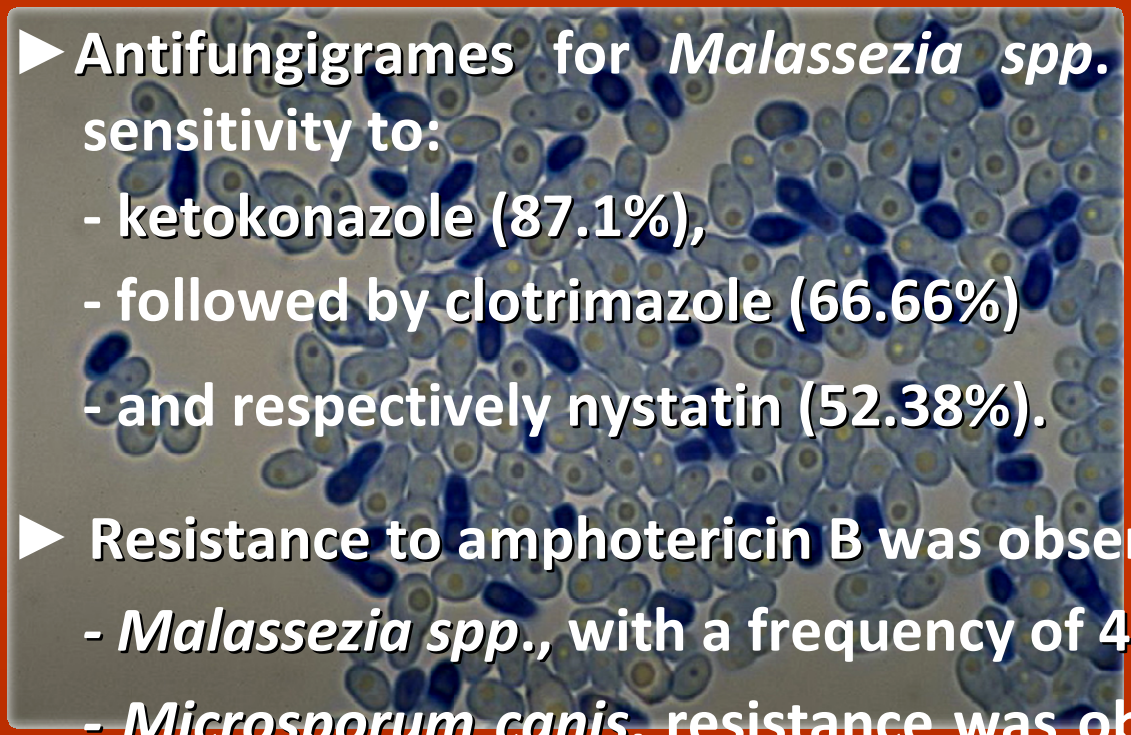
Microorganisms	Acute erythematous-ceruminous otitis		Chronic - suppurative otitis		Isolated strains	
	Nr.	%	Nr.	%	Nr.	%
Staphylococcus spp.	3	20	5	8,92	8	11,26
S. aureus	-	-	9	16,07	9	12,67
Streptococcus grup G	-	-	2	3,57	2	2,81
Pseudomonas aeruginosa	-	-	9	16,07	9	12,67
Proteus ssp.	-	-	4	7,14	4	5,63
Pateurella spp.	-	-	11	19,64	11	15,49
Malassezia spp.	5	33,33	16	28,57	21	29,57
Microsporum canis	7	46,66	-	-	7	9,85
Total	15	100	56	100	71	100
%	21,16		78,87			

- Results of positive cultures in relation to the growth of fungi, and yeasts, revealed high percentage of:
  - yeasts of the genus *Malassezia spp.* (29.57%)
  - followed by *Microsporum canis* (9.85%)

- If we refer to the clinical aspects of otitis:
  - erythematous ceruminous forms predominated in infections with *Microsporum canis* (44.66%),
  - followed by yeasts of the genus *Malassezia spp.* (33.33%).

- In chronic forms, complicated suppurative,
  - dermatophyts were absent,
  - while the yeast genus *Malassezia spp*. were present in a proportion of 28.57%.

- Of the total isolates, were isolated in:
  - pure culture microorganisms only 12.67%,
  - while in mixed cultures, the proportion was 87.32%.



- Antifungigrames for *Malassezia spp.* strains, showed high sensitivity to:
  - ketokonazole (87.1%),
  - followed by clotrimazole (66.66%)
  - and respectively nystatin (52.38%).
- Resistance to amphotericin B was observed in the case of:
  - *Malassezia spp.*, with a frequency of 42.85% and in case of
  - *Microsporum canis*, resistance was observed in clotrimazole and amphotericin B, respectively (57.14%).

Sensitivity to antifungals of *Malassezia spp*. and *Microsporum spp*. isolated of OE from the cat

Antifungals	Interpretation	*Malassezia spp.*		*Microsporum spp.*	
		Nr.	%	Nr.	%
Clotrimazole	S	14	66,66	2	28,57
	I	2	9,52	1	14,28
	R	5	23,80	4	57,14
Ketokonazole	S	18	85,71	5	71,42
	I	2	9,52	2	28,57
	R	1	4,76	-	-
Nystatin	S	11	52,38	3	42,85
	I	4	19,04	1	14,28
	R	6	28,57	3	42,85
Amphotericin B	S	8	38,09	1	14,28
	I	4	19,04	2	28,57
	R	9	42,85	4	57,14

Note:
S – Sensitive,
I – Intermediate sensitive,
R – Resistant

- Obtained data were compared with those from literature and the results of other researchers, many of them being in consonance with our results.

Conclusions

- In acute forms of otitis externa in cats, microbial flora is represented by *Microsporum canis* species dermatophyts
- In chronic forms in most cases are represented by *Malassezia*
- In the chronic complicated suppurative forms, dermatophyts were absent, while *Malassezia spp*. yeasts were isolated in association with bacterias
- Antifungal sensitivity of *Malassezia* and *Microsporum* strains isolated from cats with otitis externa was increased to ketokonazol, followed by clotrimazole