Pneumococcal infections in Romania

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Brasov Romania


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Nasopharyngeal carriage

- Gambia: 76.1 – 85.1
- Pakistan: 51.9 – 64.4%
- Philippines: 51 – 62%
- South Africa: 29.4%
- Southern Israel: 35% - 93%
- Uruguay: 15.2 – 42.1%
- Zambia: 71.9%

Nasopharyngeal colonization

with *Streptococcus pneumoniae*

in healthy infants and young children in

Brasov, Central Romania:

antibiotic resistance, serotype distribution, prevalence of nonvaccine serotypes and 7-valent pneumococcal conjugate vaccine coverage

Study objectives

1) To determine the NP colonization rates with *S. pneumoniae* among healthy children living in Brasov, Central Romania

2) To analyze the antimicrobial susceptibility and serotypes distribution of *S. pneumoniae* isolated in NP samples obtained in healthy children living in the community (with their families)

3) To analyze the antimicrobial susceptibility and serotypes distribution of *S. pneumoniae* isolated in NP samples obtained in healthy children attending a DCC in Brasov

Study objectives (2)

5) To analyze the potential coverage of the 7-valent pneumococcal conjugate vaccine (PCV7) and additional conjugated vaccines (10 and 13 valent) for *S. pneumoniae* isolated in the above mentioned patient groups

4) To compare the antimicrobial susceptibility and serotypes distribution of *S. pneumoniae* isolated in NP samples obtained in healthy children attending DCC with those of children living with their families

Patients and Methods

Study population.

- Brasov has a population of ~400,000; the Children's Hospital is the only pediatric hospital in the city, with ~3,000 deliveries/year and ~90-100 visits at the emergency room/day.
- Starting **April 1, 2008**, NP cultures for *S. pneumoniae* were performed till **January 31, 2009** in the following groups of children**:**

a.) Healthy young children aged 2-5 years attending 6 DCCs in the city of Brasov (100 children) - the samples were collected on April 17, May 3, June 6, October 12 and December 17 during 2008 and January 24, 2009. Each DCC was visited and samples were collected during one single day.

Patients and Methods (2)

b.) 100 consecutive healthy infants and young children < 5 years age visiting during the period April 2008-January 2009 the clinics of 5 GP physicians in Brasov for completion of their immunizations

c.) Healthy infants and young children < 5 years of age examined during the period April 1, 2008-January 31, 2009 at the emergency room of Children's Hospital of Brasov with a diagnosis of trauma or another non-infectious diseases (100 infants and children)

d.) Healthy infants and young children < 5 years of age hospitalized during the period April 1-January 31, 2009 at the surgery departments of Children's Hospital of Brasov for elective surgical procedures (hernias, urologic procedures, ENT procedures, eye surgery, orthopedic procedures etc.)-(100 infants and children)

Patients and Methods (2)

The stored organisms were further transported by air to **Israel to the Microbiology Laboratories of the Soroka University Medical Center in Beer-Sheva** for confirmation of the organism, determination of antimicrobial susceptibility, serotyping and molecular typing by pulsed field gel electrophoresis (PFGE).

	Daycare Centers	Elective Surgery	Immunization Clinics	Emergency Room	Total
No. patients	**100**	**100**	**100**	**100**	**400**
Males	42	58	50	47	197
Age (months)					
Mean ± SD	42.5 ±13.2	33.6±17.2	9.4±8.2	40.1±14.7	31.4±19.0
Range (months)	2-60	1-60	2-49	2-60	1-60
Median	45	36	8	45	35
Previous antibiotic treatment					
During last 48 h	8	27	13	12	60
During last month w/o last 48 h	17	17	31	28	93
Previous AOM history*					
No previous AOM	83	77	87	83	330/384 (86)**
1-2 previous episodes	12	11	11	14	48/384 (13)**
≥ 3 previous episodes	5	2	0		6/384 (2)**

Colonization data on various age group among 400 infants and children enrolled at 4 sampling centers

Age (months)	Daycare Center (n =100)	Elective surgery (n = 100)	Immunization clinics (n = 100)	Emergency room (n=100)	Total (n = 400)
Total colonized	71	38	62	34	205/400 (51)
< 12	1/2 (50)*	9/18 (50)	53/90 (59)	7/8 (88)	70/118 (59)
13-24	11/11 (100)	12/17 (71)	6/7 (86)	3/9 (33)	32/44 (73)
25-36	13/16 (82)	5/17 (29)	0/0	4/18 (22)	22/51 (43)
37-48	33/35 (94)	7/23 (30)	2/2 (100)	14/37 (38)	56/97 (58)
49-60	13/36 (36)	5/25 (20)	1/1	6/28 (21)	25/90 (28)

Distribution of the 205 *S. pneumoniae* isolates: most frequently isolated serotypes (in descending order)

Serotype	Total	% of total no. of isolates
23F	52	25
6B	31	15
19F	26	13
14	20	10
6A	16	8
19A	11	5
11A	6	3
9V	5	3
15B/C	4	2
15A	3	1
Other	31	15
7-valent vaccine serotypes	135	66
TOTAL	205	100%

Serotype distribution according to 7, 10 and 13-valent pneumococcal conjugate vaccines among the children sampled at each center

Serotype	Daycare Centers n=100	Elective Surgery n=100	Immunization Clinics n=100	Emergency Room n=100	Total n=205
4	0	0	0	0	0
6B	8 (11)*	7 (18)	12 (19)	4 (12)	31 (15)
9V	1 (1)	0	3 (5)	1 (3)	5 (2)
14	8 (11)	4 (11)	5 (8)	3 (9)	20 (10)
18C	0	0	0	1 (3)	1 (1)
19F	9 (13)	5 (13)	10 (15)	2 (6)	26 (13)
23F	14 (20)	10 (26)	17 (27)	11 (32)	52 (25)
Total 7-valent vaccine	**40 (56)**	**26 (68)**	**47 (76)**	**22 (65)**	**135 (66)**
1	**0**	**0**	**0**	**0**	**0**
5	**0**	**0**	**0**	**0**	**0**
7F	**0**	**0**	**0**	**0**	**0**
Total 10-valent vaccine	**40 (56)**	**26 (68)**	**47 (76)**	**22 (65)**	**135 (66)**
3	**2 (3)**	**0**	**0**	**0**	**2 (1)**
6A	**5 (7)**	**5 (13)**	**3 (5)**	**3 (9)**	**16 (8)**
19A	**9 (13)**	**0**	**1 (2)**	**1 (3)**	**11 (5)**
Total 13-valent vaccine	**56 (79)**	**31 (81)**	**51 (83)**	**26 (77)**	**164 (80)**
Total *S. pneumoniae* isolates	**71**	**38**	**62**	**34**	**205**

205 *S. pneumoniae* isolates: Antibiotic susceptibilities according to various antibiotic classes

	Susceptible	Intermediate	Resistant	Total resistant (I +R)
Penicillin	35 (17%)*	**85 (41.5%)**	**85 (40.5%)****	**170/205 (83%)**
Ceftriaxone	169 (82%)	**3 (2%)**	**33 (16%)**	**36/205 (18%)**
Erythromycin	(38%)	**-**	**127 (62%)**	**127/205 (62%)**
TMP/SMX	70 (34%)	**8 (4%)**	**127 (62%)**	**135/205 (66%)**
Clindamycin	88 (43%)	**-**	**117 (57%)**	**117/205 (57%)**
Tetracyclin	98 (48%)	**17(8%)**	**90 (44%)**	**107/205 (52%)**
Chloramphenicol	196 (96%)	**-**	**9 (4%)**	**9/205 (4%)**

I=intermediate resistant; R=high resistant

* in parentheses : percentage of all 205 *S. pneumoniae* isolates

** 40/205 (19.5%) with MIC ≥ 4 µg/ml (very high resistance

Conclusions

1. This study documented high nasopharyngeal colonization rates among infants and children in the Brasov area of Central Romania
2. The colonization rates with *S. pneumoniae* were age and sampling center-related, with the highest rates recorded in infants and children <2 years of age and those attending DCC
3. The most frequent isolated serotypes were PCV7-included serotypes 23F, 6B, 19F and 14. The PCV7-associated serotypes 6A and 19A represented (together) 13% of all isolates
4. PCV7-serotypes represented 66% of all isolates; the PCV10 coverage was identical with that provided by PCV7. Eighty percent of the isolates were included in the PCV13
5. 83% and 18% of all isolates were nonsusceptible to penicillin and ceftriaxone, respectively; 40.5% and 16% of all isolates were highly-resistant to penicillin and ceftriaxone

6. The nonsusceptibility rates to erythromycin, TMP/SMX, tetracyclin and clindamycin were very high (>50%) of all isolates) and similar between the 4 sampling centers; the majority of *S. pneumoniae* isolates were susceptible to chloramphenicol
7. Multidrug resistance was extremely high (67% for ≥3 antibiotic classes and 43% for ≥5 antibiotic classes)
8. The majority of of serotypes 6A, 6B, 14, 19A, 19F and 23F were nonsusceptible to penicillin; high resistance to penicillin was recorded in >45% of serotypes 6B, 14, 19F and 23F ; high resistance to ceftriaxone was found only among serotype 23F isolates
9. Of the 52 23F-*S. pneumoniae* isolates, the majority had unusually high MIC values to penicillin and ceftriaxone
10. Assuming a similarity between the carried isolates and those causing disease, the findings reported in this study make the empirical treatment of infections potentially caused by *S. pneumoniae* in the Brasov area of central Romania extremely problematic

Table 1

Streptococcus pneumoniae carriage rate according to the age group

Age group (*n*)	Carriers, *n* (%)	Carriage rate 95% CI
0–11 months (186)	31 (16.7)	12.0–22.7
12–35 months (751)	162 (21.6)	18.8–24.5
36–60 months (1063)	312 (29.4)	26.7–32.2
Total	505 (25.3)	23.4–27.2

CI, confidence interval.

M.Luminos et al: Int J of Inf Dis.2014;29:169-173. Nasopharyngeal carriage of Streptococcus pneumoniae in children in Romania before the introduction of pneumococcal conjugate vaccine into national immunozation prgramme a national multi centre cross sectional observational study.

Table 2
Serotype distribution in the three age groups: 0–11, 12–35, and 36–60 months

Serotype	0–11 months (n = 29), n	12–35 months (n = 141), n	36–60 months (n = 283), n	Total (n = 453), n (%)
6A	3	14	34	51 (11.2)
6B	6	19	35	60 (13.2)
6 non-A, non-B	1	10	26	37 (8.2)
14	1	10	14	25 (5.5)
15	3	12	16	31 (6.8)
19A	4	10	17	31 (6.8)
19F	6	32	54	92 (20.3)
19 non-A, non-F	1	6	26	33 (7.3)
23F	1	5	11	17 (3.8)
23 non-F	0	7	23	30 (6.6)
Other PCV[a]	1	0	4	5 (1.1)
Other non-PCV[b]	2	16	22	39 (8.6)

PCV, pneumococcal conjugate vaccine.

M.Luminos et al: Int J of Inf Dis.2014;29:169-173. Nasopharyngeal carriage of Streptococcus pneumoniae in children in Romania before the introduction of pneumococcal conjugate vaccine into national immunozation prgramme a national multi centre cross sectional observational study.

Table 3

Serotype distribution related to PCV serotypes (number (percentage) and 95% confidence interval)

	Serotypes included in vaccine			NVT[a]
	PCV7	PCV10	PCV13	
0–11 months (n=31)	15 (48.4%) 32.0–65.2	15 (48.4%) 32.0–65.2	23 (74.2%) 56.7–86.3	8 (25.8%) 13.7–43.3
12–35 months (n=162)	66 (40.7%) 33.5–48.4	68 (41.9%) 34.6–49.7	93 (57.4%) 49.7–64.8	69 (42.6%) 35.2–50.3
36–60 months (n=312)	118 (37.8%) 32.6–43.3	118 (37.8%)	174 (55.7%)	138 (44.3%) 38.8–49.8
	M. Luminos et al. / International Journal of Infectious Diseases 29 (2014) 169–173			
Total (n=505)	199 (39.4%) 35.2–43.7	201 (39.8%) 35.6–44.1	290 (57.4%) 53.1–61.7	215 (42.6%) 38.3–46.9

PCV, pneumococcal conjugate vaccine.

[a] NVT: serotypes not included in the PCV13 vaccine or non-typeable *S. pneumoniae*.

M.Luminos et al: Int J of Inf Dis.2014;29:169-173. Nasopharyngeal carriage of Streptococcus pneumoniae in children in Romania before the introduction of pneumococcal conjugate vaccine into national immunization prgramme a national multi centre cross sectional observational study.

Microbiologic Characteristics and Pneumococcal Conjugate Vaccine (PCV) Coverage of Acute Otitis Media (AOM) in Children <5 Years of Age in Brasov, Central Romania, in the Pre-vaccination Era (2009-2011)

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Introduction (1)

- Acute otitis media (AOM) is the most frequent bacterial disease of childhood, affecting millions of children worldwide.
- The most common causative agents in AOM are *Streptococcus pneumoniae, non typeable Haemophilus influenzae (NTHi), Moraxella catarrhalis and Streptococcus pyogenes.*
- Together *Streptococcus pneumoniae and NTHi* account for 60-80% of the AOM pathogens.
- **Antibiotic resistance** is high in pneumococcal AOM with penicillin and amoxicillin – nonsusceptible strains accounting for **30-70%.**
- The **highest nonsusceptibility** is found for vaccine-serotypes **6A, 6B, 9V, 14, 19A and 23F**

Klein JO. Vaccine 2000;19:S2-8.
Grevers G. Int J Ped Otorhinol 2010; 74:572-77

Aims of the study

- to assess the overall distribution of otopathogens and their antibiotic resistance
- PCV coverage of S.pneumoniae in middle ear fluid (MEF) isolates in Brasov, Central Romania

Patients and methods

- We conducted a prospective epidemiological study between January 1st 2009 throughout December 31 2011, at the Childrens Hospital from Brasov, central part of Romania.
- The overall population is around 400000 inhabitants.
- Children's Hospital is the only medical center providing medical care for infants and children in the city.
- The study protocol was approved by the institutional review board of the Transilvania University from Brasov.

Patients and procedures

- The study patients were infants and children under the age of 5
- The diagnoses of otitis media was done when:

- patients presented with symptoms and physical findings consistent with AOM (fever, irrritability, tugging of the ear, redness and biling of the tympanic membrane withblurring of its anatomic landmarks)

- acute illness lasting under 7 days.

- Bullging of the tympanic membrane was present in all cases were tympanocentesis was performed.
- Culture specimens were obtained by either tympanocentesis or collection of pus from draining ear

Results

- During this study period 212 consecutive children under the age of 5 years were enrolled.
- 56.6% of them were males
- Mean age (±SD) was 18.0±14.2 months.
- 46.6% episodes occured in patients under 12 months old and 64.2% occured in children less than 2 years.
- 111 were culture positive.
- Children with culture positive MEF were older than children with culture negative MEF (20.6±15.2 months vs. 15.4±12.6 months p=0.008).

Falup-Pecurariu O et al. Pneumococcal acute otitis media infants and children in central Romania 2009-2011 microbiological characteristics and potential coverage by pneumococcal conjugate vaccine. Int J Inf. Dis. 2013; 17(9):e702-6.

- Tympanocentesis and spontaneous otorrhea were recorded in 142 and 70 patients respectively.
- There was no difference recorded in spontaneous perforation between children with culture positive and children with culture negative MEF.

Falup-Pecurariu O et al. Pneumococcal acute otitis media infants and children in central Romania 2009-2011 microbiological characteristics and potential coverage by pneumococcal conjugate vaccine. Int J Inf. Dis. 2013; 17(9):e702-6.

AOM microbiology

Pathogen	# of episodes
S. pneumoniae	78 (70.3)
H. influenzae	23 (20.7)
S. pyogenes	5 (4.5)
M. catarrhalis	2 (1.8)
S. pneumoniae + H. influenzae	3 (2.7)
Culture positive	111
Culture negative	101
Total	212

Falup-Pecurariu O et al. Pneumococcal acute otitis media infants and children in central Romania 2009-2011 microbiological characteristics and potential coverage by pneumococcal conjugate vaccine. Int J Inf. Dis. 2013; 17(9):e702-6.

- Reliable information regarding previous antibiotic treatment was available in 68/111 (60.2%) culture positive patients.
- 42.6% culture positive patients received previous antibiotic treatment compared to 33.7% of the culture negative patients.
- *S.pneumoniae* was higher in patients previously untreated with antibiotics compared with patients treated with antibiotics 71.7% vs 48.3%.

Falup-Pecurariu O et al. Pneumococcal acute otitis media infants and children in central Romania 2009-2011 microbiological characteristics and potential coverage by pneumococcal conjugate vaccine. Int J Inf. Dis. 2013; 17(9):e702-6.

Antibiotic susceptibility

- Antibiotic susceptibilty and serotyping was performed in 48/81 S.pneumoniae isolates.
- **93.8%** were **nonsusceptible to penicillin** and had a MIC value of above 2.0µg/ml
- 77.1% of the isolates had ceftriaxone MIC values ≥0.5µg/ml.
- The non susceptibility rates to TMP/SMX, erythromycin, clindamycin were: **75%, 58.3%, 35.4%.**

Falup-Pecurariu O et al. Pneumococcal acute otitis media infants and children in central Romania 2009-2011 microbiological characteristics and potential coverage by pneumococcal conjugate vaccine. Int J Inf. Dis. 2013; 17(9):e702-6.

S.pneumoniae serotype distribution

Serotype	# of episodes	MDR
19F	29.20%	92.90%
23F	16.70%	87.50%
6B	16.70%	100%
14	12.50%	
19A	6.20%	66.70%
6A	4.10%	50%

Falup-Pecurariu O, Leibovitz E, Mercas A, Bleotu L, Zavarache C, Porat N, Dagan R, Greenberg D – Manuscript submmitted

Comparison of *S. pneumoniae* isolated from patients with community acquired pneumonia versus acute otitis media

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Background

- PCV vaccine not being on the Romanian national immunization program (NIP) offers a unique opportunity to study the serotype distribution of Pnc in mucosal diseases and to study the antibiotic resistance pattern in a specific frame time in our region.
- It is possible that there are differences between Pnc serotypes carried and antibiotic resistance strains in AOM compared with CAP, probably related to a predilection to organ specificity

Aim

The aims of the presented study were: to compare the serotype distribution and the antibiotic resistance patterns between two mucosal diseases: AOM and CAP in children <5 years of age in a non-PCV vaccinating country

Patients and methods

- Is was a prospective study in Children's Hospital of Brasov central part of Romania. Children younger than 5 years of age admitted to the hospital due to CAP respectively AOM from 2009 through 2014 were enrolled.
- Acute otitis media was defined as consistent symptoms and physical findings for AOM(fever, tubbing of the ear, irritability and bulgging of the tympanic membrane with blurring of its anatomic landmarks) that were lasting for less than 7 daysSi
- Community acquired pneumonia diagnosed was based on patients' history, clinical signs, and laboratory tests findings, such as complete white blood cells count (WBC) and chest radiograph

RESULTS

- A total number of 117 patients were enrolled, 29 children with AOM and 88 with CAP at which we performed serotyping
- The mean age and male gender were not different between groups (**TABLE 1**)
- Saturation in room air was statistically different with a lower saturation in the CAP group as it was expected, also the respiratory rate was statistically different with a $p<0.0001$ (**TABLE 1**)
- Pneumococcus was the most common pathogen followed by *H. influenzae* in CAP (**Table 2**)

Tabel 1.Demographic and clinical characteristic of children with AOM *vs.* CAP from Brasov, Romania

	AOM (N=29)	CAP (N=88)	P
Age (mean±SD)	25.1 ±24.01	27.01 ±28.46	0.78
Male (n, %)	19.0 ±65.5	47 ±53.41	0.25
Rural (n, %)	13 ±44.8	51 ±57.95	0.21
Crowded house (n, %)	10 ±62.50	43 (51.81)	0.43
Passive smoking (n, %)	14 (60.87)	51 (61.45)	0.95

* all differences to total group count are missing data

Table 2: Bacteriology characteristic of children with AOM vs. CAP from Brasov, Romania

	AOM N=391	CAP N=559	P
S. pneumoniae (n, %)	129 (34.4)	200 (35.8)	0.53
H. influenzae (n, %)	60 (15.3)	86 (15.4)	0.98
M. catarrhalis (n, %)	3 (0.8)	38 (6.8)	<0.0001
Group A Streptococcus (n, %)	19 (4.8)	1 (0.2)	<0.0001

Table 3: Serotypes distribution in children with AOM vs. CAP from Brasov, Romania

SEROTYPE (n, %)	AOM N=29	CAP N=88	P
23F	5 (17.2)	20 (22.7)	0.61
19F	14 (48.2)	15 (17.0)	**0.012**
19A	2 (6.9)	6 (6.8)	0.98
14	3 (10.3)	5 (5.6)	0.42
6B	1 (3.4)	17 (19.3)	**0.068**
15A	2 (6.9)	3 (3.4)	0.44
6A	1 (3.4)	17 (19.3)	**0.068**
3	0 (0)	2 (2.2)	0.41
Other	2 (6.9)	20 (22.7)	0.104
PCV7 serotypes *	24 (82.7)	60 (68.1)	0.54
PCV13 serotypes**	26 (89.6)	72 (81.8)	0.77
Non-PCV13 serotypes	3 (10.3)	16 (18.1)	0.39

*4,6B,9V,14,18C,19F, and 23F
**1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F

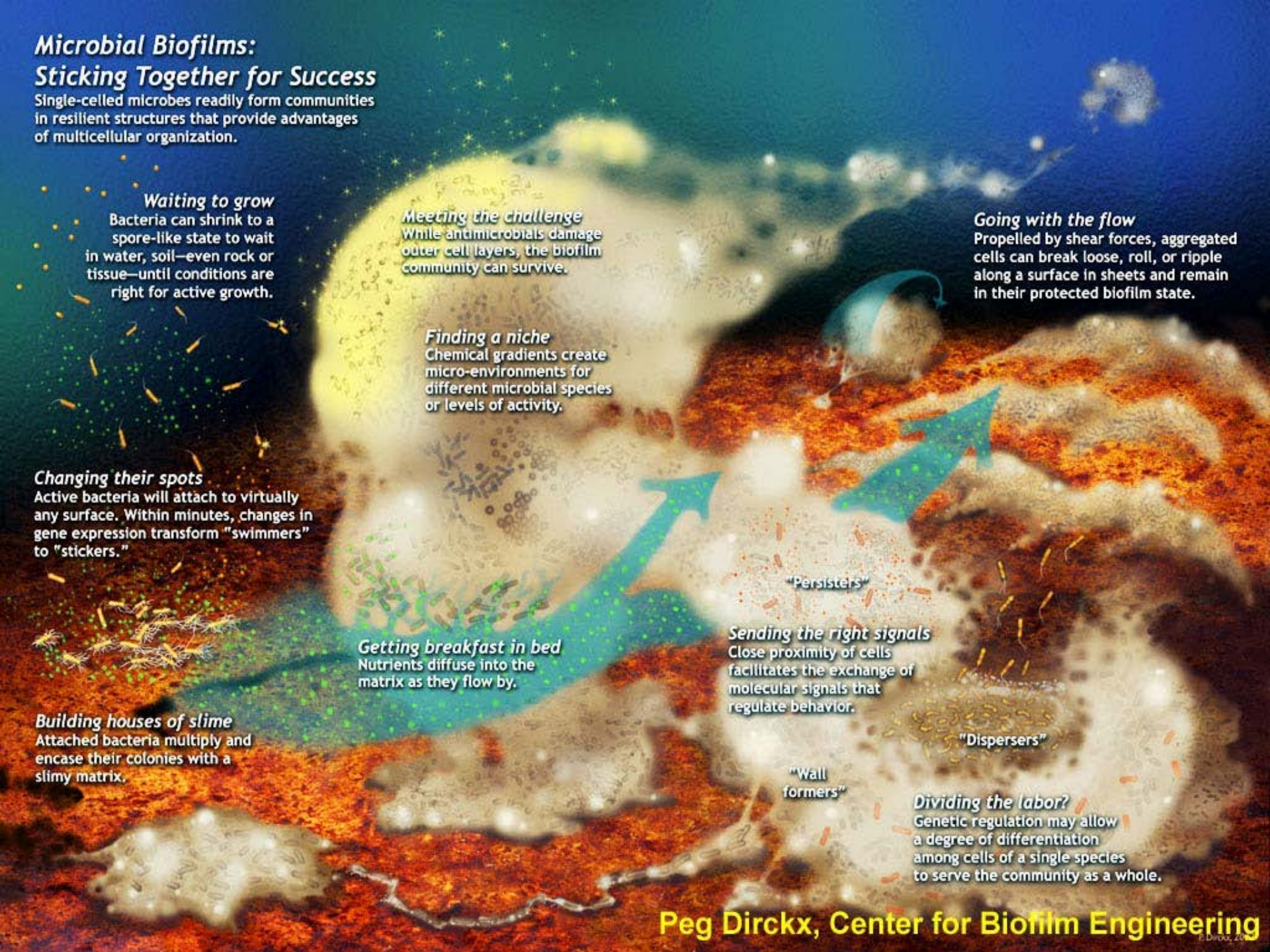
Table 4: Antibiotic non-susceptible rates in children with AOM *vs.* CAP from Brasov, Romania

Antibiotic	AOM N=29	CAP N=88	P
Oxacillin	28 (96.55)	76 (87.36)	0.71
Erythromycin	23 (79.31)	61 (69.32)	0.67
Tetracyclin	19 (65.52)	44 (50)	0.33
Chloramphenicol	0	3 (3.41)	0.31
TMP/SMX*	29 (100)	74 (84.09)	0.02
Clindamycin	18 (62.07)	54 (61.36)	0.94
Penicillin M.I.C. (µgr/ml) (mean±SD)	8.0 ±27.59	21.0 ±24.14	0.82
Ceftriaxon M.I.C. (µgr/ml) (mean±SD)	6.0 (20.69)	15.0 ±17.24	0.107

M. I. C. minimal inhibitory concentration
TMP/SMX-Trimethoprim/sulfamethoxazole

CONCLUSION

- ✓ In both mucosal diseases mostly affected were males and children living in crowded houses.
- ✓ The most frequently encountered serotypes in AOM and CAP are 14, 6B,19F, 23F.
- ✓ The most common serotypes isolated from children with CAP <5 years were: 23F, 6B, 19F, 19A, 14
- ✓ Serotypes 6A and 6B were more common among CAP isolates while serotype 19F was more common in AOM isolates


Microbial Biofilms:
Sticking Together for Success
Single-celled microbes readily form communities in resilient structures that provide advantages of multicellular organization.
Waiting to grow
Bacteria can shrink to a spore-like state to wait in water, soil—even rock or tissue—until conditions are right for active growth.
Meeting the challenge
While antimicrobials damage outer cell layers, the biofilm community can survive.
Going with the flow
Propelled by shear forces, aggregated cells can break loose, roll, or ripple along a surface in sheets and remain in their protected biofilm state.
Finding a niche
Chemical gradients create micro-environments for different microbial species or levels of activity.
Changing their spots
Active bacteria will attach to virtually any surface. Within minutes, changes in gene expression transform "swimmers" to "stickers."
"Persisters"
Sending the right signals
Close proximity of cells facilitates the exchange of molecular signals that regulate behavior.
Getting breakfast in bed
Nutrients diffuse into the matrix as they flow by.
"Dispersers"
"Wall formers"
Building houses of slime
Attached bacteria multiply and encase their colonies with a slimy matrix.
Dividing the labor?
Genetic regulation may allow a degree of differentiation among cells of a single species to serve the community as a whole.
Peg Dirckx, Center for Biofilm Engineering

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