

Antibiotic resistance determinants in oral bacteria

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Purpose of the study

To identify and characterize both known and unknown antibiotic resistance determinants from oral biofilms using a metagenomic approach



Long term goal of the study

To better understand the mechanisms by which the resistance determinants develop, disseminate, and persist among oral bacterial populations



Oral disease (infections)

- In 2005, Americans made about 500 million visits to dentists, and an estimated \$84 billion was spent on dental services (CDC).
- Surgeon general's report (2000)
 - ❖ Dental caries (tooth decay) is the single most common chronic childhood disease--5 times more common than asthma and 7 times more common than hay fever.
 - ❖ Most adults show signs of periodontal diseases. Severe periodontal disease (measured as 6 millimeters of periodontal attachment loss) affects about 14 percent of adults aged 45 to 54.
 - ❖ Twenty-three percent of 65- to 74-year-olds have severe periodontal disease.



Chronic oral infections and systemic disease

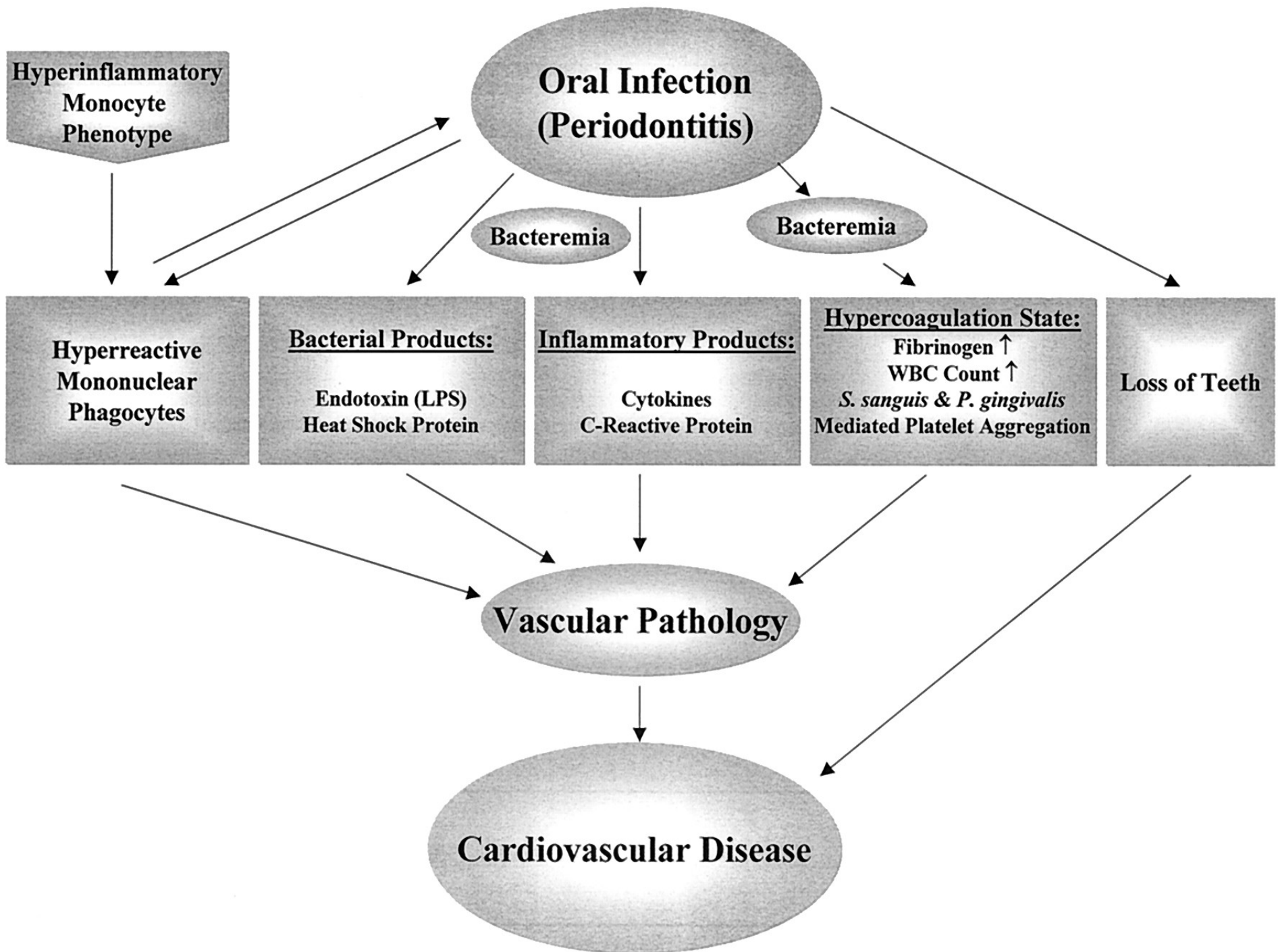
- Oral infections may affect the course and pathogenesis of a number of systemic diseases including:
 - ❖ Diabetes
 - ❖ Cardiovascular disease
 - ❖ Premature birth (pre-term, low-birth-weight)
 - ❖ Respiratory disease



Possible mechanisms

- Spread of infection from the oral cavity as a result of transient bacteremia
- Injury from the circulating oral microbial toxins
- Inflammation caused by bacterial component-virulence determinants
- Subgingival dental plaque as reservoirs of gram-negative bacteria, resulting in inflammatory mediators



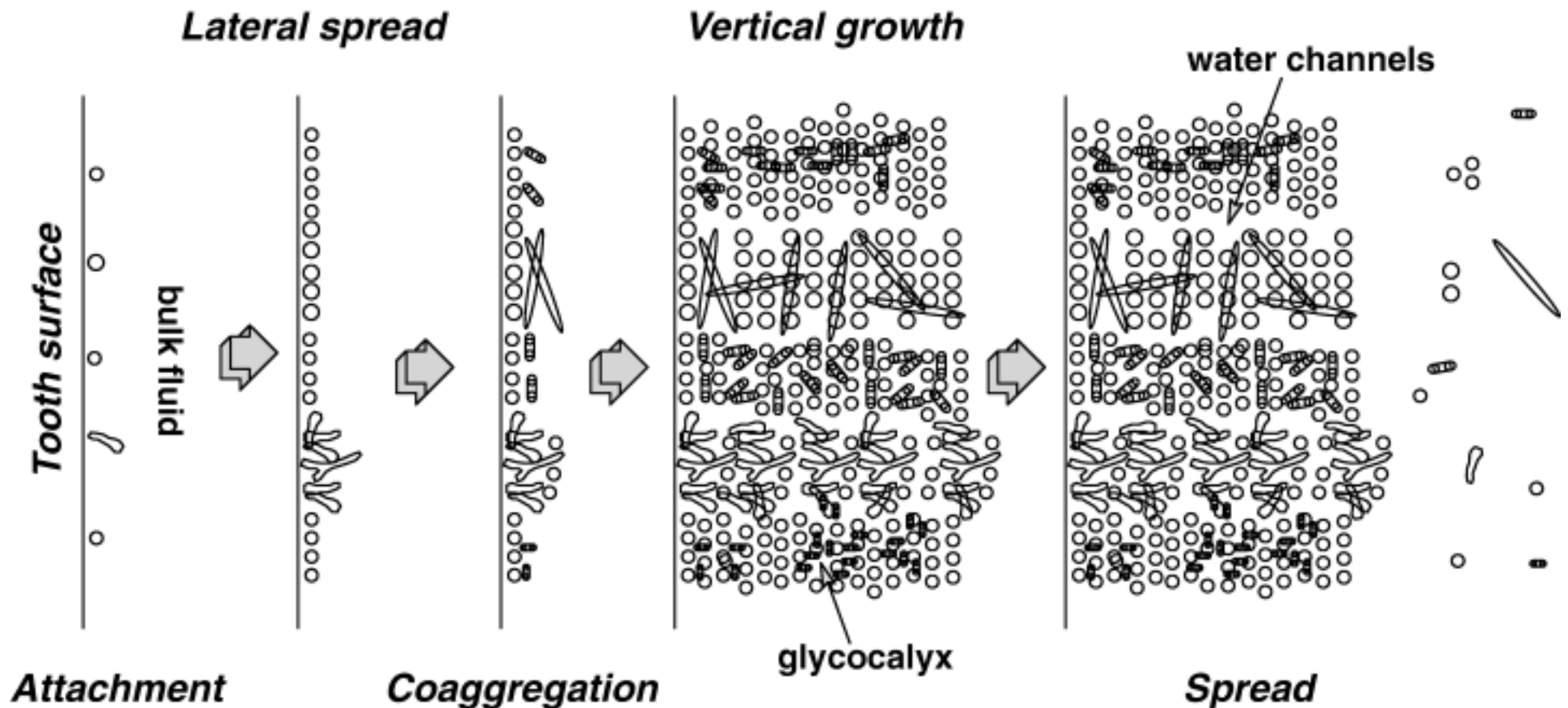


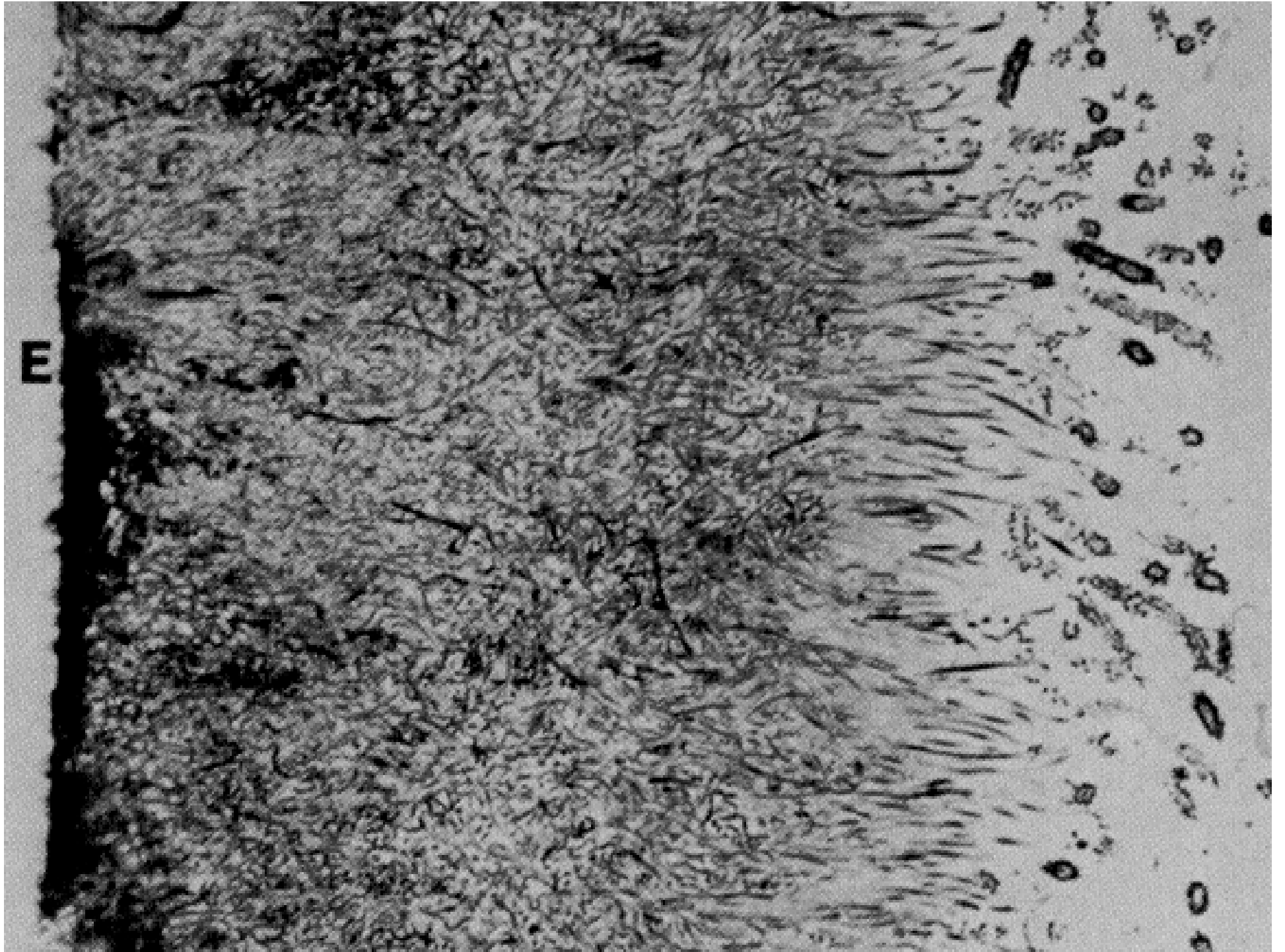
Oral biofilms

- Consists of ~700 bacterial species
 - ❖ 400 in subgingival dental plaque biofilm
 - ❖ About half are cultivable
- Dental plaque biofilm
 - ❖ Etiological agent of dental caries and periodontal disease

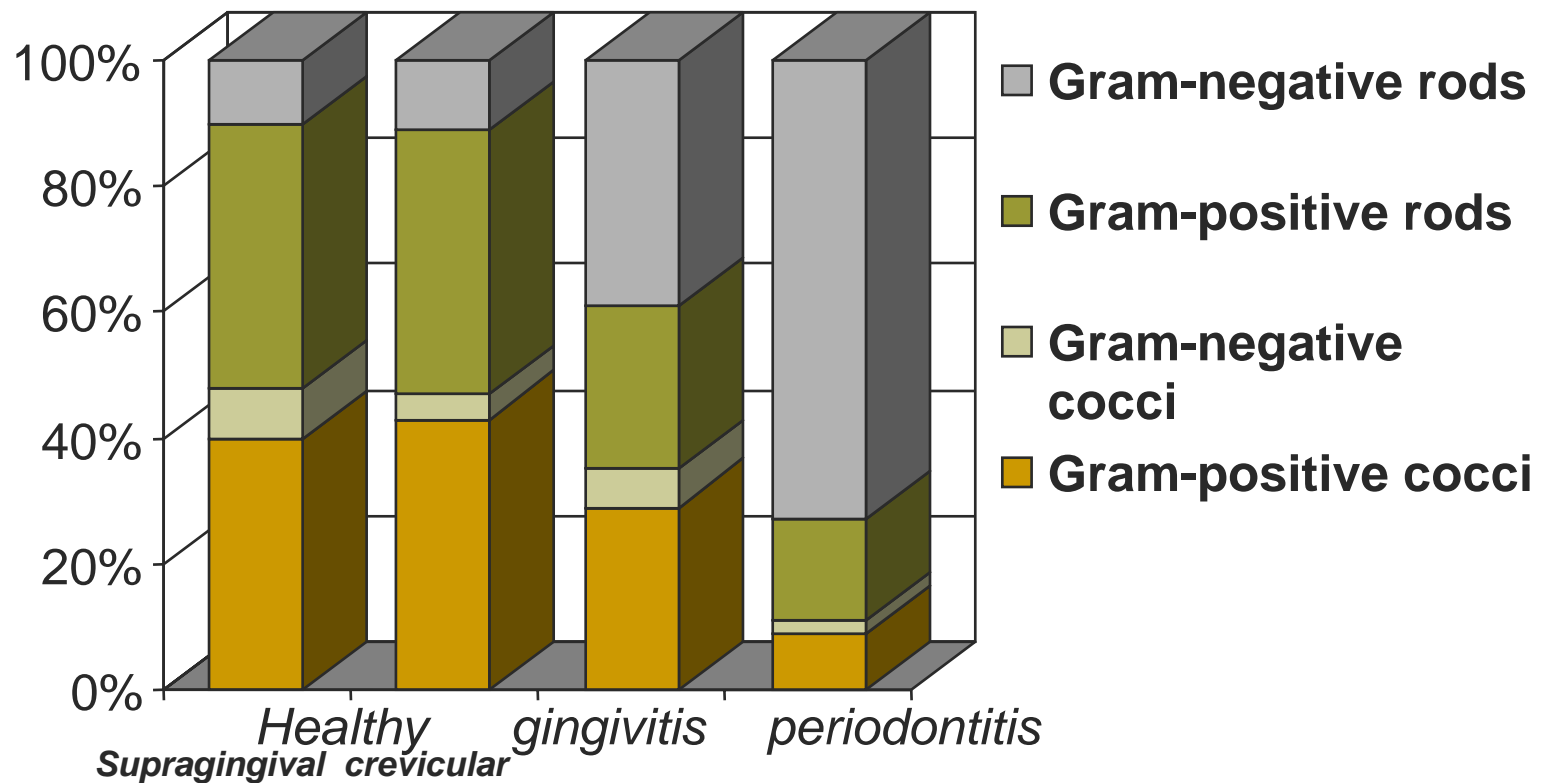


Development of dental plaque biofilm

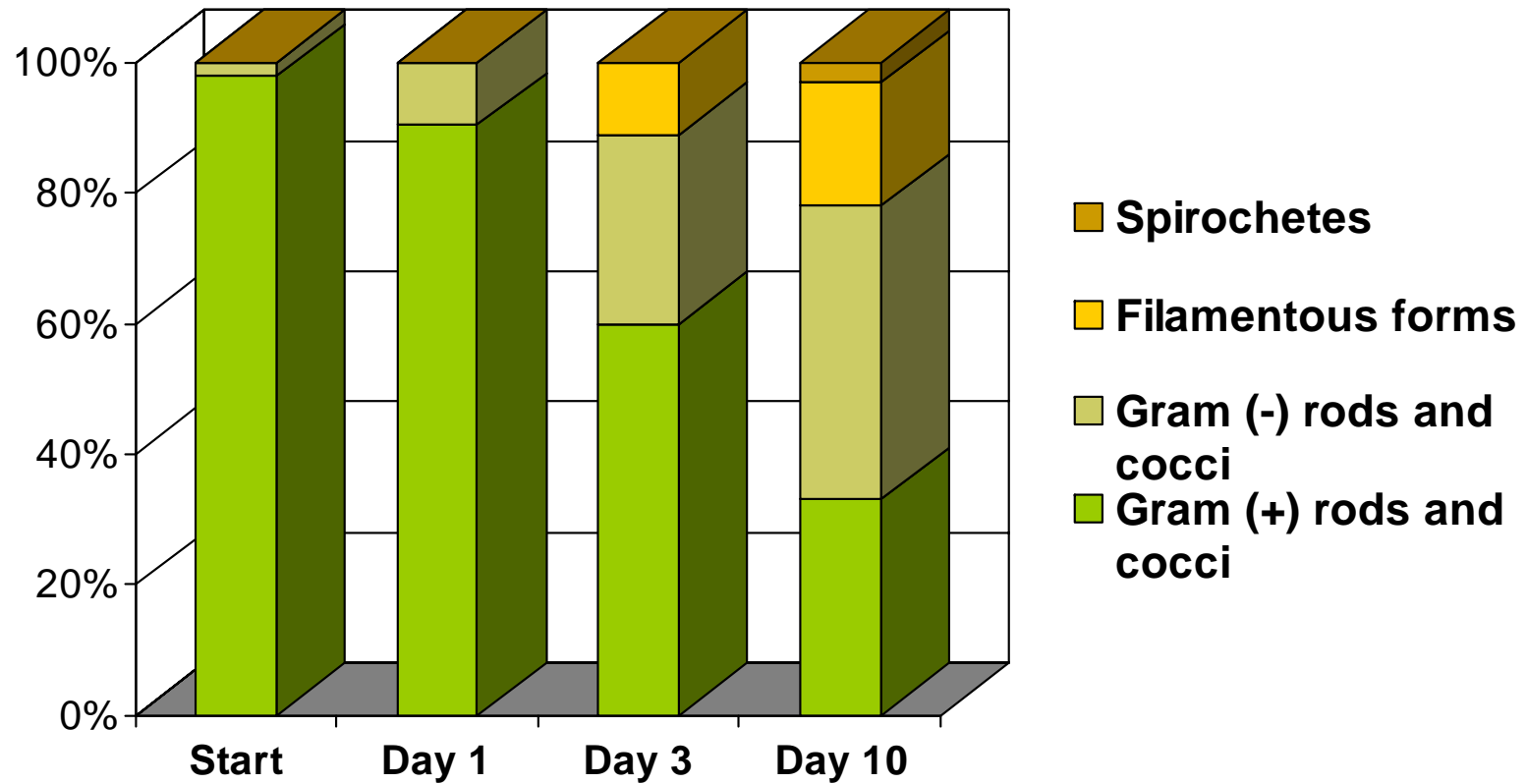




Microbiota associated with periodontal health, gingivitis, and periodontal disease

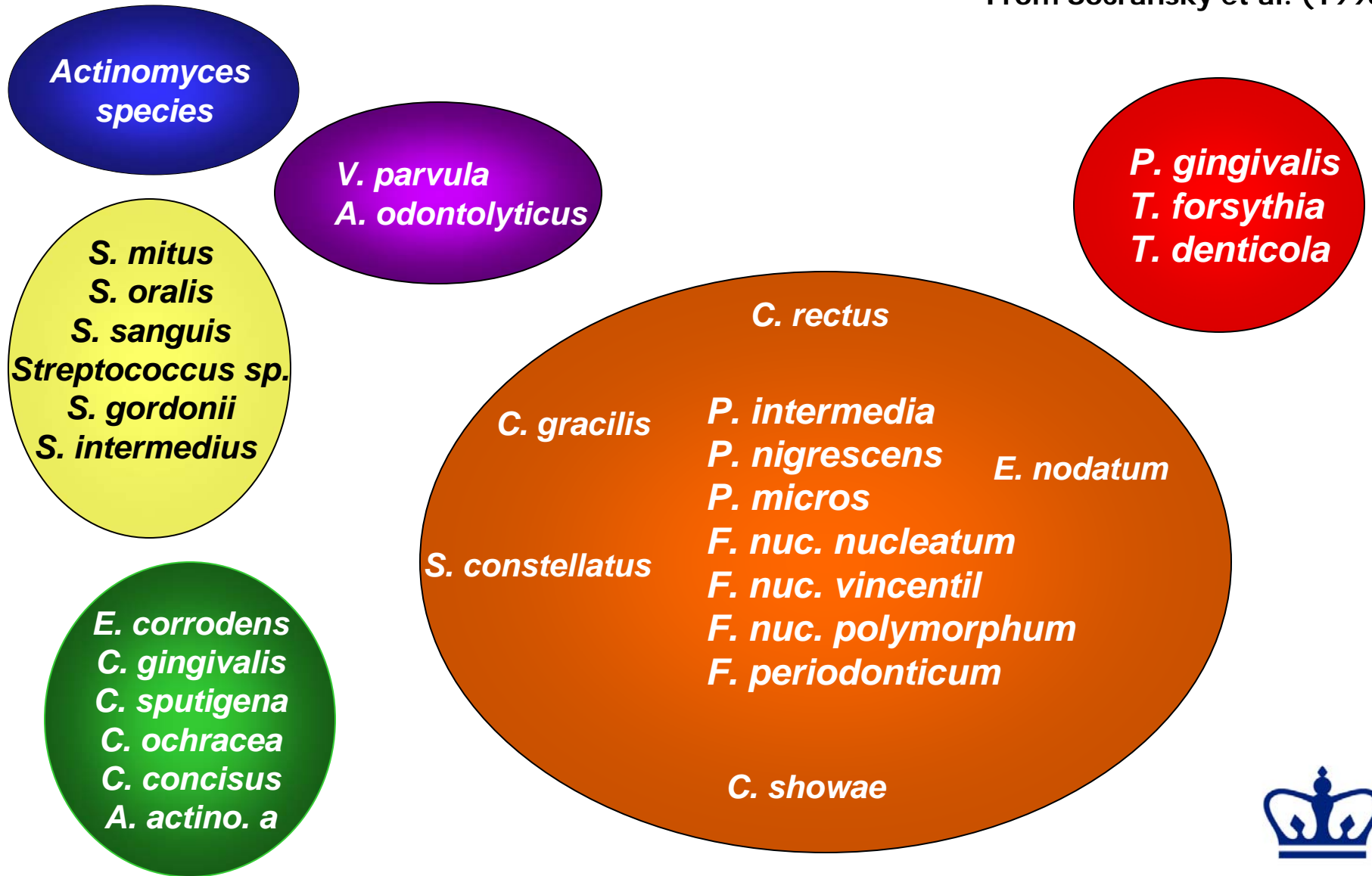


Plaque reformation after cleaning



Subgingival microbial complex

-- From Socransky et al. (1998)



Antibiotic resistance in oral bacteria

- Diversity of oral biofilm colonizers
- Mostly commensals
 - ❖ A breeding ground for antibiotic resistance – evidence of horizontal gene transfer
- Ecological difference in health vs disease-associated biofilm
 - ❖ Difference in resistance?
 - ❖ Disease-associated organisms possess more mobile element – facilitating gene transfer



Metagenomic analysis

Environmental sample

Metagenomic library construction

Extract DNA → **Clone** → **Transform into a host bacterium (e.g. *E. coli*)**

Metagenomic library

Metagenomic analysis

screen for particular sequences by PCR or hybridization

random sequencing

screen for expression of particular phenotypes

Specific Aims

- To identify both known and unknown antibiotic resistance determinants
- To globally assess the diversity and distribution of antibiotic resistance determinants among different bacterial populations



Significance

- Allows identification and characterization of resistance determinants from uncultivable bacteria
- Provides a global view of the genetic diversity of antimicrobial resistance among different microbial populations in health and disease
- May lead to discovery of novel resistance mechanisms
- May lead to development of new antibiotic drugs inhibiting novel resistance mechanisms

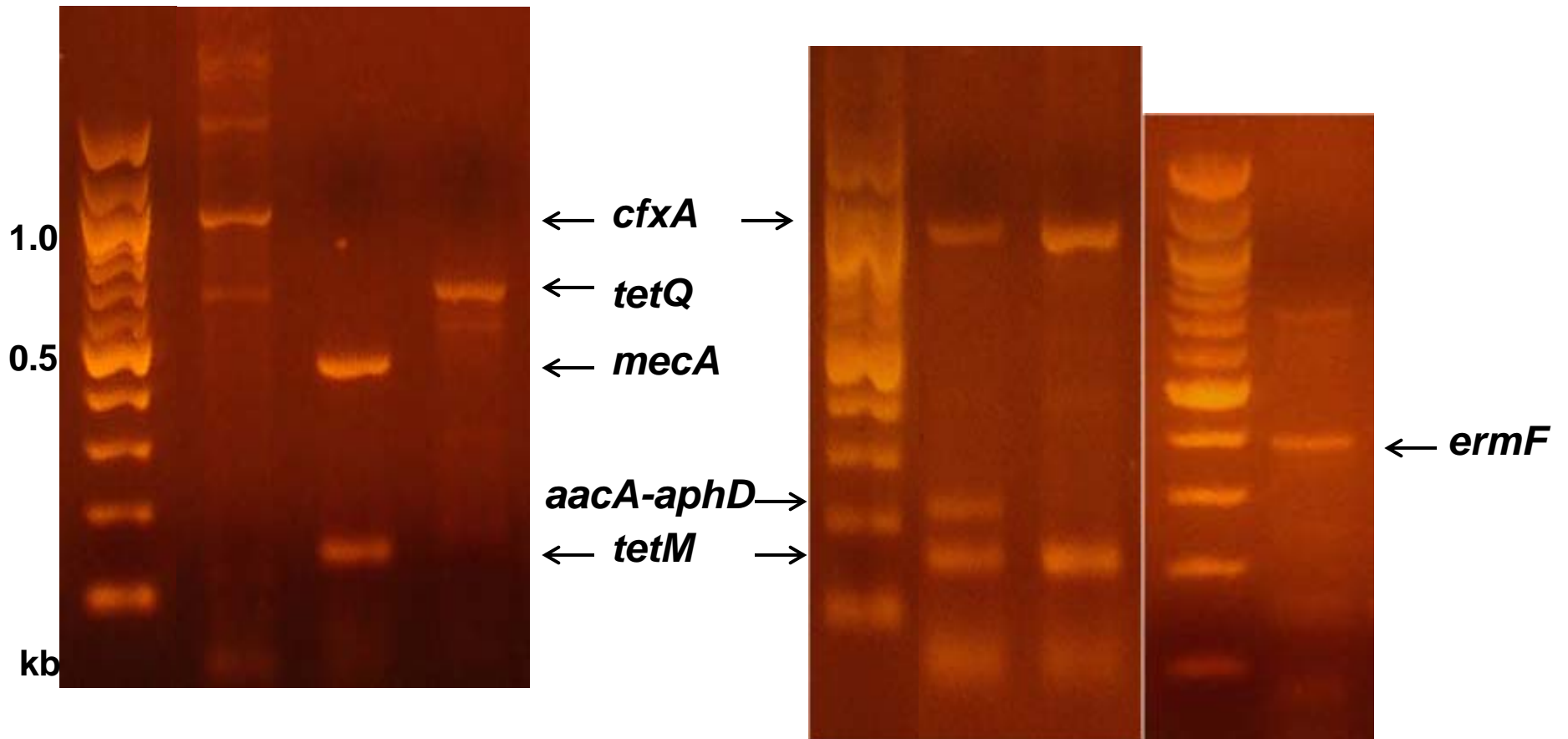


Methods

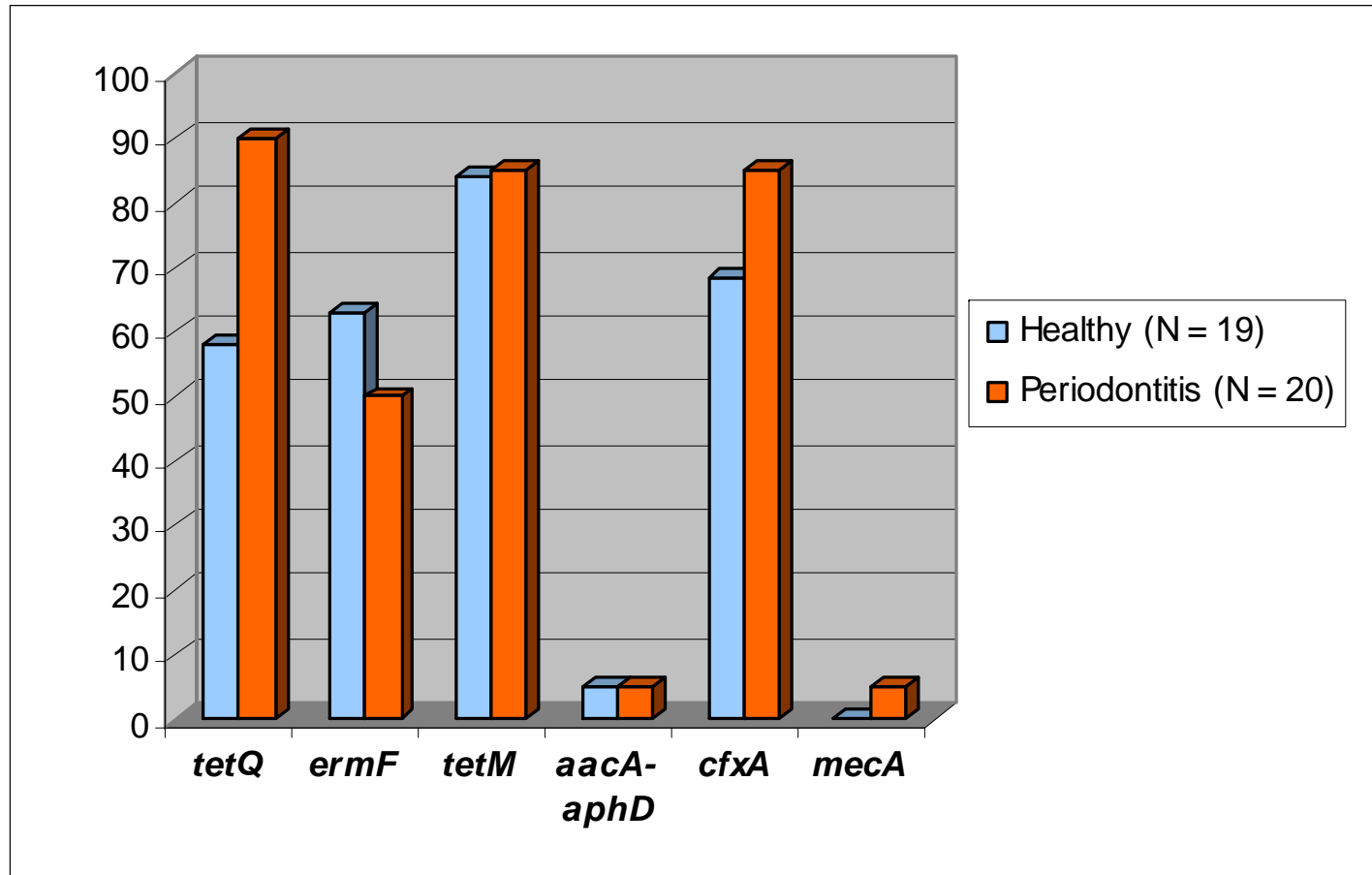
- Collection of microbial samples from different niches
- Extraction of bacterial genomic DNA from the samples
- Construction of genomic library by inserting DNA into the *E. coli* vector
- Screening of the library for resistance based on expression or the known sequences
- Identification of already known or novel resistance determinants



Identification of resistance genes by PCR



Prevalence of selected resistance determinants in subgingival dental plaque biofilms



Sequencing and comparison of *tetQ*

Prevotella intermedia tetQ, *B. fragilis tetA(Q)3*, *P. ruminicola tetQ*

```
1 50
H14 TATTGTGGCCCAGTAAGGGTTGGGTGGTACTTCGATCTGAATAATCTTAT
P27 TATTGTGGCCCAGTAAGGGTTGGGTGGTACTTCGATCTGAATAATCTTAT
P30 TATTGTGGCCCAGTAAGGGTTGGGTGGTACTTCGATCTGAATAATCTTAT

51 100
H14 TGACCTTTTTTATAGGTCGTTCTTTGTAGATAGTCTTGATCTCATCAAAA
P27 TGACCTTTTTTATAGGTCGTTCTTTGTAGATAGTCTTGATCTCATCAAAA
P30 TGACCTTTTTTATAGGTCGTTCTTTGTAGATAGTCTTGATCTCATCAAAA

. . . . .

501 550
H14 CTCGAAGTCTCAGACTTCCGTCAATTATTTAAGAAAACCTCTTTTATGC
P27 CTCGAAGTCTCAGACTTCCGTCAATTATTTAAGAAAACCTCTTTTATGC
P30 CTCGAAGTCTCAGACTTCCGTCAATTATTTAAGAAAACCTCTTTTATGC

551 600
H14 CCTTGGGGTCATGCTCTATCTTATAGAGATAAGCTGAAAGTCTGTTTGA
P27 CCTTGGGGTCATGCTCTATCTTATAGAGATAAGCTGAAAGTCTGTTTGA
P30 CCTTGGGGTCATGCTCTATCTTATAGAGATAAGCTGAAAGTCTGTTTGA
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Sequencing and comparison of *tetM*

P27, P32: *Streptococcal* conjugative transposon Tn916 *tetM*

P28: *Streptococcal* conjugative transposon Tn1545 *tetM*

```
1 50
P28 TGACGGCGAGGTCACGAAACGGATAATACGCTTTTAGAACGTCAGAGA
P27 TGACGTGGAAGGTCACGAGGACGGATAATACGCTTTTAGAACGTCAGAGA
P32 TGACGTGGAAGGTCACGAGGACGGATAATACGCTTTTAGAACGTCAGAGA
```

```
51 100
P28 GGAATTACAATTCAGACGGCGATAACCTCTTTTCAGTGGAAAATACTAA
P27 GGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAA
P32 GGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAA
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101 130
P28 GGTGAACATCATAGACACGCCAGGACATAT
P27 GGTGAACATCATAGACACGCCAGGACATAT
P32 GGTGAACATCATAGACACGCCAGGACATAT
```



Sequencing and comparison of *cfxA*

Prevotella intermedia class A β -lactamase precursor gene (*cfxA2*)

```
1 50
H16 GCTGATATATGCGCAACATATTGTGACGCTTGTGATTCATTTCCCTTGAA
P31 GCTGATATATGCGCAACATATTGTGACGCTTGTGATTCATTTCCCTTGAA
H17 GCTGATATATGCGCAACATATTGTGACGCTTGTGATTCATTTCCCTTGAA

51 100
H16 ATCCTTAACAAATACCGCTAAGGTATAACTGATATTATTAGGCAGACATA
P31 ATCCTTAACAAATACCGCTAAGGTATAACTGATATTATTAGGCAGACATA
H17 ATCCTTAACAAATACCGCTAAGGTATAACTGATATTATTAGGCAGACATA

. . . . .

601 650
H16 ACTAAGGTATCAAGTGAAATTCCTTTATTGTCAAAGTCATTACAAAGAGC
P31 ACTAAGGTATCAAGTGAAATTCCTTTATTGTCAAAGTCATTACAAAGAGC
H17 ACTAAGGTATCAAGTGAAATTCCTTTATTGTCAAAGTCATTACAAAGAGC

651 700
H16 TAATGCCTGATGAACCTTAAACACACTCATCATAGGATAAACTCTTAT
P31 TAATGCCTGATGAACCTTAAACACACTCATCATAGGATAAACTCTTAT
H17 TAATGCCTGATGAACCTTAAACACACTCATCATAGGATAAACTCTTAT

701
H16 TATTGACCTTAACCGTATCTCTGTTATTAACAATA
P31 TATTGACCTTAACCGTATCTCTGTTATTAACAATA
H17 TATTGACCTTAACCGTATCTCTGTTATTAACAATA
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Sequencing and comparison of *ermF* and *aacA-aphD*

- *ermF* (P26 = H14 = H12)
 - ❖ 100 % match to transposon Tn4351 with insertion element IS 4351 (*B. fragilis* R plasmid) encoding *ermF*
- *aacA-aphD* (H13)
 - ❖ 100 % match to *S. aureus*, *Enterococcus*, *S. faecalis aacA-aphD*

