Ecosystem's Oxygen Deficiency and Selection of Antimicrobial Resistance Genes in a One Health Perspective

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Abstract

Bacteria must face and adapt to a variety of physicochemical conditions in the environment and during infection. A key condition is the concentration of dissolved oxygen, proportional to the partial pressure of oxygen (PO_2), which is extremely variable among environmental biogeographical areas and also compartments of the human and animal body. Here, we sought to understand if the phenotype of resistance determinants commonly found in Enterobacterales can be influenced by oxygen pressure. To do so, we have compared the MIC in aerobic and anaerobic conditions of isogenic *Escherichia coli* strains containing 136 different resistance genes against 9 antibiotic families. Our results show a complex landscape of changes in the performance of resistance genes in anaerobiosis. Certain changes are especially relevant for their intensity and the importance of the antibiotic family, like the large decreases in resistance observed against ertapenem and fosfomycin among bla_{VIM} ß-lactamases and certain *fos* genes, respectively; however, the $bla_{\mathrm{OXA\text{-}48}}$ ß-lactamase from the clinically relevant pOXA-48 plasmid conferred 4-fold higher ertapenem resistance in anaerobiosis. Strong changes in resistance patterns in anaerobiosis were also conserved in *Klebsiella pneumoniae*. Our results suggest that anaerobiosis is a relevant aspect that can affect the action and selective power of antibiotics for specific AMRs in different environments.

Keywords: Dissolved oxygen, anaerobiosis, antimicrobial resistance, antimicrobial resistance genes, environmental pharmacokinetics, integrons, antibiotics.

44 Antimicrobial resistance (AMR) is one of the major health concerns of the 21st century
45 globally, according to the World Health Organization (WHO)[1,2]. The dissemination of AMR
46 occurs in the global human-animal-environmental continuum, as considered in the One
47 Health perspective[3,4], yet the physicochemical conditions of these different compartments are
48 diverse. In humans and animals AMR frequency is mostly determined by antibiotic
49 consumption, following selection of bacteria harboring antibiotic resistance genes (ARGs),
50 frequently harbored in mobile genetic platforms such as plasmids and integron cassettes
51 (ARCs)[5,6]. Additionally, growing antibiotic pollution in the environment also exerts a
52 significant selective pressure on AMR bacteria[7]. The environmental pharmacokinetics of
53 antibiotics remain poorly explored, and the laboratory conditions in which the susceptibility
54 of pathogenic bacteria is tested (based on MIC, minimal inhibitory concentration), strongly
55 differ from the variable ecological compartments, in animal body sites, or in the environment
56 at large[8].
57 One of these unmet conditions in laboratory tests is the variability of oxygen
58 concentrations. Here, we sought to understand if the detoxifying performance of ARGs
59 commonly found in Enterobacterales can be influenced by dissolved oxygen (directly
60 proportional to the PO2, the partial pressure of oxygen) in such a way that it contributes in
61 various degrees to the selection of AMR in aerobic/anaerobic conditions.
62 To investigate the impact of anaerobiosis on resistance genes, we used mainly our pMBA
63 collection[9] that is composed of 136 isogenic strains of *E. coli* MG1655, each containing a
64 particular ARC as the first cassette of a class 1 integron borne by the pMBA plasmid vector[10].
65 The collection contains resistance genes against 9 antimicrobial families covering a broad
66 variety of mechanisms. We have also included other clinically relevant AMR plasmids and
67 genes. We have determined the MIC levels of every member in the collection in the presence

68 and absence of oxygen (Figs. 1B and 1C). As a control, a strain containing the empty pMBA
69 vector was included. To rid our analysis of the potential effects of anaerobiosis on the
70 bacterium's resistance -and not in the gene's function- the *performance* of an AMR gene is
71 measured as the fold-increase in resistance it confers compared to the empty pMBA in a given
72 environmental condition. Additionally, the strain containing pMBA reproduced the effect of
73 anaerobiosis found in *E. coli* without pMBA, allowing to rule out confounding effects of the
74 vector (Fig. 1A). Our data show that several ARCs varied their antimicrobial resistance effect
75 according to oxygen availability. For example, the aminoglycoside resistance cassette
76 *aacA56* (Figs. 1B and 1C) increases 8-fold the MIC to amikacin in the absence of oxygen,
77 enhancing the effect of energy-dependent antibiotic reduced uptake. In addition, some
78 families of ARCs showed opposite trends in resistance in anaerobiosis: different variants of
79 the metallo-ß-lactamase bla_{VIM} and $bla_{\mathrm{IMP\text{-}2}}$ were less efficient in anaerobiosis, reducing their
80 performance against ertapenem between 8 and 64-fold on average (Figs. 1B and 1C). A
81 similar decrease in resistance was observed for several fosfomycin resistance genes (*fos*) in
82 anaerobiosis -with the exception of *fosE,* that showed a 4-fold increase (Figs. 1B). Because
83 both bla_{VIM} and *fos* encode metalloenzymes (that use respectively Zn^{2+} and Mn^{2+} as
84 cofactors) it is tempting to speculate that metal coordination is being affected in anaerobiosis.

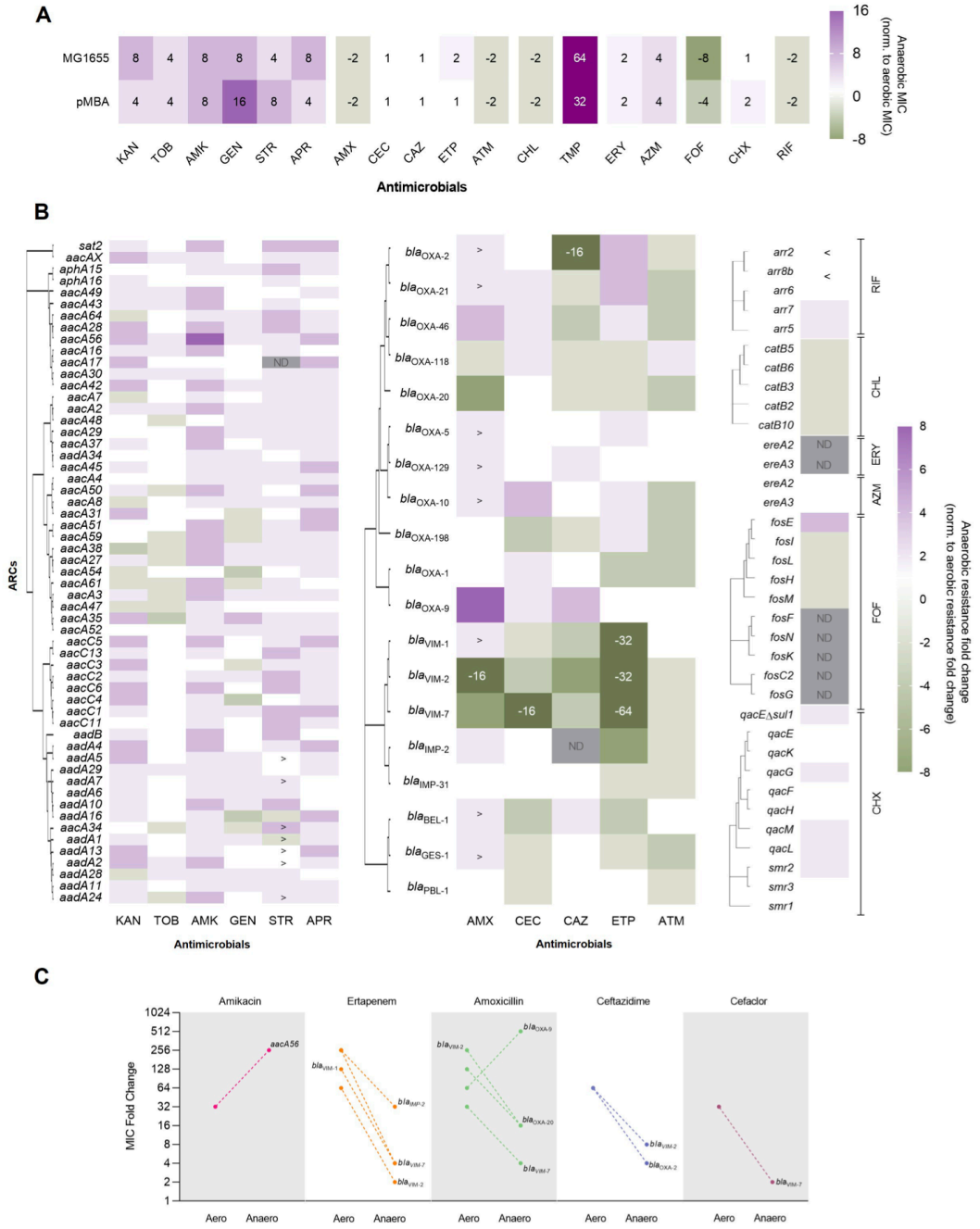


Figure 1. Anaerobiosis affects ARC's function in *E. coli*. A) Heatmap showing the fold-change in resistance of *E. coli* MG1655 -with and without pMBA- between anaerobic and aerobic conditions against 18 antimicrobials (KAN: kanamycin, TOB: tobramycin, AMK: amikacin, GEN: gentamicin, STR: streptomycin, APR: apramycin, AMX: amoxicillin, CEC: cefaclor, CAZ: ceftazidime, ETP: ertapenem, ATM: aztreonam, CHL: chloramphenicol, TMP: trimethoprim, ERY: erythromycin, AZM: azithromycin, FOF: fosfomycin, CHX:

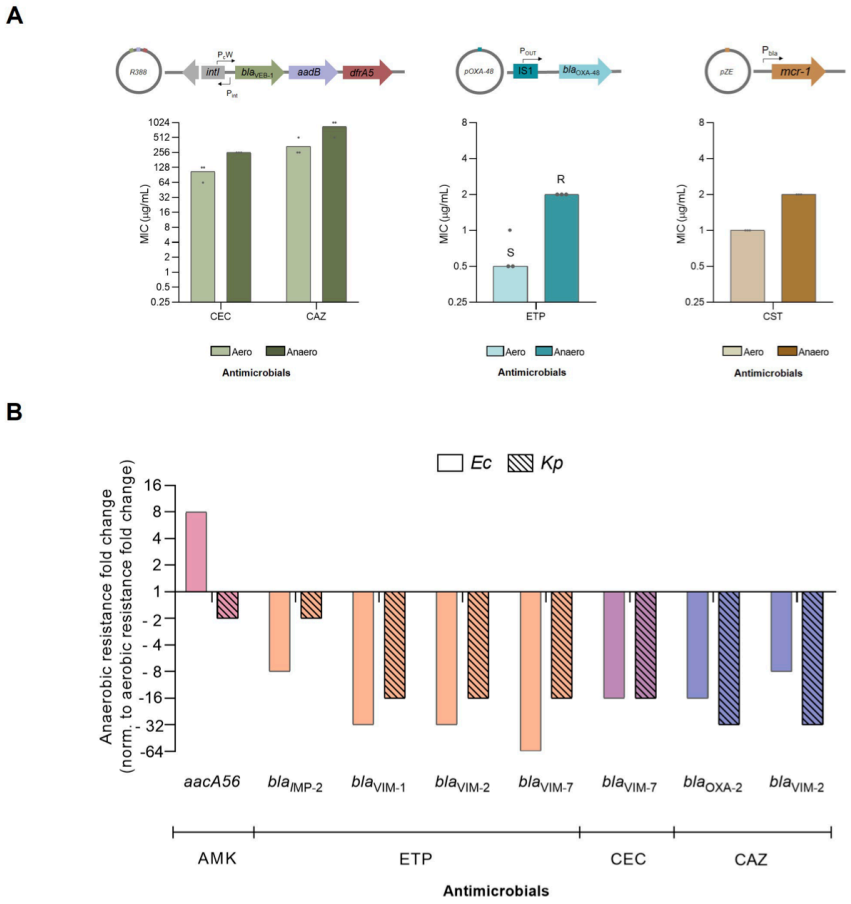
91 chlorhexidine, RIF: rifampicin). Numbers in each square indicate the fold-change in resistance between
92 anaerobic and aerobic conditions. **B)** Heatmap showing the resistance profile of each ARC in anaerobiosis,
93 normalized by its effect in aerobic conditions. Purple indicates that an ARC confers a higher resistance increase
94 (compared to pMBA) in anaerobic conditions than in aerobic conditions, while green represents a lower
95 resistance increase (compared to pMBA) in anaerobiosis compared to aerobiosis. Red squares depict cases for
96 which a precise MIC could not be determined (strains showing growth at the highest concentration of antibiotic
97 tested) in any of both conditions. Symbols > and < are used to indicate strains that grew at the highest
98 concentration of antibiotic tested in anaerobic or aerobic conditions respectively. A hierarchical clustering tree
99 showing protein sequence similarity is shown in the left side of each panel for (from left to right)
100 aminoglycoside resistance cassettes, ß-lactam resistance cassettes, other ARCs. **C)** MIC fold change (compared
101 to pMBA) in aerobiosis and anaerobiosis of the subset of ARCs, showing at least an 8-fold difference between
102 conditions. Points represent the mode of at least three biological replicates.

103

104 At least 8 ARCs showed strong changes in their performance in anaerobiosis (defined as fold
105 changes ≥ 8-fold between conditions) (Fig. 1C). This can be of clinical relevance, as
106 carbapenemases inactivating last resource antimicrobial agents, such as bla_{VIMs} and $bla_{\text{IMP-2}}$,
107 confer lower resistance levels in anaerobiosis. Our data suggests that bacteria carrying these
108 metallo-ß-lactamase genes could become susceptible to drugs such as ertapenem in anaerobic
109 compartments of the animal or human body (such as the large intestine), including infection
110 sites or in particular environmental spaces. Yet, the conventional antibiogram would yield an
111 ertapenem resistance phenotype, wrongly preventing clinicians from using a possibly
112 successful drug. The opposite trend is observed for other ß-lactamases, such as OXA-9 and
113 OXA-48 ß-lactamases. Indeed, plasmid pOXA-48 confers resistance to ertapenem in *E. coli*
114 under anaerobic conditions but the strain is considered susceptible to this antibiotic under
115 aerobic conditions. A similar phenomenon is observed for plasmid R388[11] containing $bla_{\text{VEB-}}$

116 1, where resistance to ß-lactam antibiotic ceftazidime increases in anaerobiosis (Fig. 2A). The
117 resistance profile of an *mcr-1* did not show relevant changes (≥ 4-fold change) in anaerobic
118 conditions (Fig 2A).

119 To test if these results are conserved in other Enterobacterales, we selected the ARCs showing
120 ≥ 8-fold change between conditions in *E. coli* and introduced them in strain ATCC23357 of
121 *Klebsiella pneumoniae*. The trends in resistance changes were similar between both species
122 (Fig. 2B).



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124 **Figure 2. Anaerobiosis modulates antimicrobial susceptibility across different species. A)** MIC of *E. coli*
125 MG1655 harboring clinically relevant resistance genes and plasmids, against several antimicrobials. Bars
126 represent the mode of three biological replicates (black dots). Light and dark colored bars represent aerobic and

anaerobic measures respectively. S and R letters stand for susceptible and resistant according to EUCAST clinical breakpoints. A scheme illustrating the genetic background of each ARG is displayed above each graph. **B)** Comparison between anaerobic MIC fold change normalized to aerobic MIC fold change of $pMBA_{ARC}$-containing *E. coli* and *K. pneumoniae*. The effect of oxygen concentration on ARCs is similar in both species. MIC fold change is calculated as the mode of at least six biological replicates. Non-patterned and patterned columns represent data retrieved from *E. coli* and *K. pneumoniae* respectively.

In this work we show how oxygen availability, as an example of physico-chemical conditions of ecosystems can influence AMR. Our results have implications in clinical treatments but also in environmental settings. Indeed, in natural water bodies the amount of dissolved oxygen depends on the depth of the water column and the action of winds, waves, photosynthetic organisms, temperature, and salinity. Water bodies are anthropogenically polluted with antibiotics and antibiotic-resistant bacteria, many of them originating from human and animal microbiota[12]. Hence, oxygen availability can influence selection of AMR organisms in the environment, in particular in sewage and sludge water treatment plants, that are frequently based on treatments decreasing dissolved oxygen[13]. Altogether, this means that the possibility of modifying the effect on the AMR genes is ecologically and geographically dependent, and AMR surveillance research on the relation of oxygen and the abundance of AMRs should probably be developed in the near future[14]. Finally, we should mention the consequences of global warming, increasing bacterial growth but reducing the availability of dissolved oxygen in different biogeographical scenarios, thus altering the environmental selection of AMR[15].

Conflict of Interest

The authors have no conflicts of interest to declare.

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Data Availability

Data sharing not applicable to this article as no datasets other than what is shown in figures were generated or analysed during the current study.

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