Supplementary Appendix

Bacterial infection, antibiotic use and COVID-19: Lessons from the intensive care unit

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This supplementary appendix is intended to be read with the manuscript and provides a more detailed account of the methodology followed to classify cultured organisms as pathogens or contaminants and to de-duplicate results. The findings at each step are shown. Antimicrobial use and resistance to the antibiotics most frequently used in our intensive care unit are also discussed.

Classification of Culture Results

For all patients admitted to the Tygerberg Hospital COVID-19 Intensive Care Unit (ICU) between 26 March 2020 and 31 August 2020, we accessed the results for all blood cultures, tracheal aspirates and urine cultures performed. Table S1 shows the number of patients with positive cultures for pathogens by site and overall, together with the timing of such cultures. “Early” cultures are those performed within 48 hours of ICU admission, which “late” cultures were performed after 48 hours.

Culture site	Early (<48 hr)	Late (>48 hr)	Time (days)
Blood culture	9	56	7.5
Urine culture	5	14	6.5
Tracheal aspirate culture	6	36	7.1
Overall	20	73	5.9

Table S1: Number of patients with positive culture for pathogen by site and overall, and mean time in days from admission to intensive care unit to first positive culture.

Table S2 shows the raw frequencies of all isolates that we found. Figure S1 shows the raw distribution of the most common organisms during the course of the pandemic and by patient day in ICU.

Organism	Blood Culture		Tracheal Aspirate		Urine Culture	
	Early	Late	Early	Late	Early	Late
Gram negative organisms						
Acinetobacter baumannii	0	45	1	34	0	2
Klebsiella pneumoniae	0	6	1	6	0	2
Enterobacter cloacae	1	6	0	1	0	0
Klebsiella oxytoca	0	6	0	1	0	0
Pseudomonas aeruginosa	0	5	0	2	0	0
Serratia marcescens	0	5	0	1	0	0
Stenotrophomonas maltophilia	0	5	1	0	0	0
Escherichia coli	0	0	0	0	3	2
Proteus mirabilis	0	2	0	0	0	1
Chryseobacterium indologenes	0	2	0	0	0	0
Morganella morganii	0	2	0	0	0	0
Pseudomonas fluorescens	0	2	0	0	0	0
Haemophilus influenzae	0	0	0	1	0	0
Gram positive organisms						
Coagulase negative staphylococci	38	17	0	0	0	0
Bacillus species	7	12	0	0	0	0
Enterococcus faecalis	1	7	0	0	2	4
Enterococcus faecium	0	3	0	0	0	3
Staphylococcus epidermidis	2	4	0	0	0	0
Corynebacterium species	1	2	0	0	0	0
Staphylococcus hominis	1	0	0	0	0	0
Staphylococcus saccharolyticus	0	1	0	0	0	0
Micrococcus species	1	0	0	0	0	0
Anaerobes						
Mycobacterium tuberculosis	0	0	1	1	0	0
Clostridium perfringens	0	1	0	0	0	0
Bacteroides caccae	0	0	1	0	0	0
Yeasts/fungi						
Candida albicans	0	2	6	5	6	12
Candida glabrata	1	2	0	1	2	4
Candida tropicalis	0	0	0	0	0	2
Candida parapsilosis	1	0	0	0	0	0
Other candida	0	0	0	0	0	2
Aspergillus fumigatus	0	0	1	0	0	0

Table S2: Raw frequency of all cultured pathogens and contaminants by culture site and timing of culture during intensive care unit admission. “Early” implies culture obtained within the first 48 hours of ICU admission while “late” refers to cultures obtained after 48 hours.

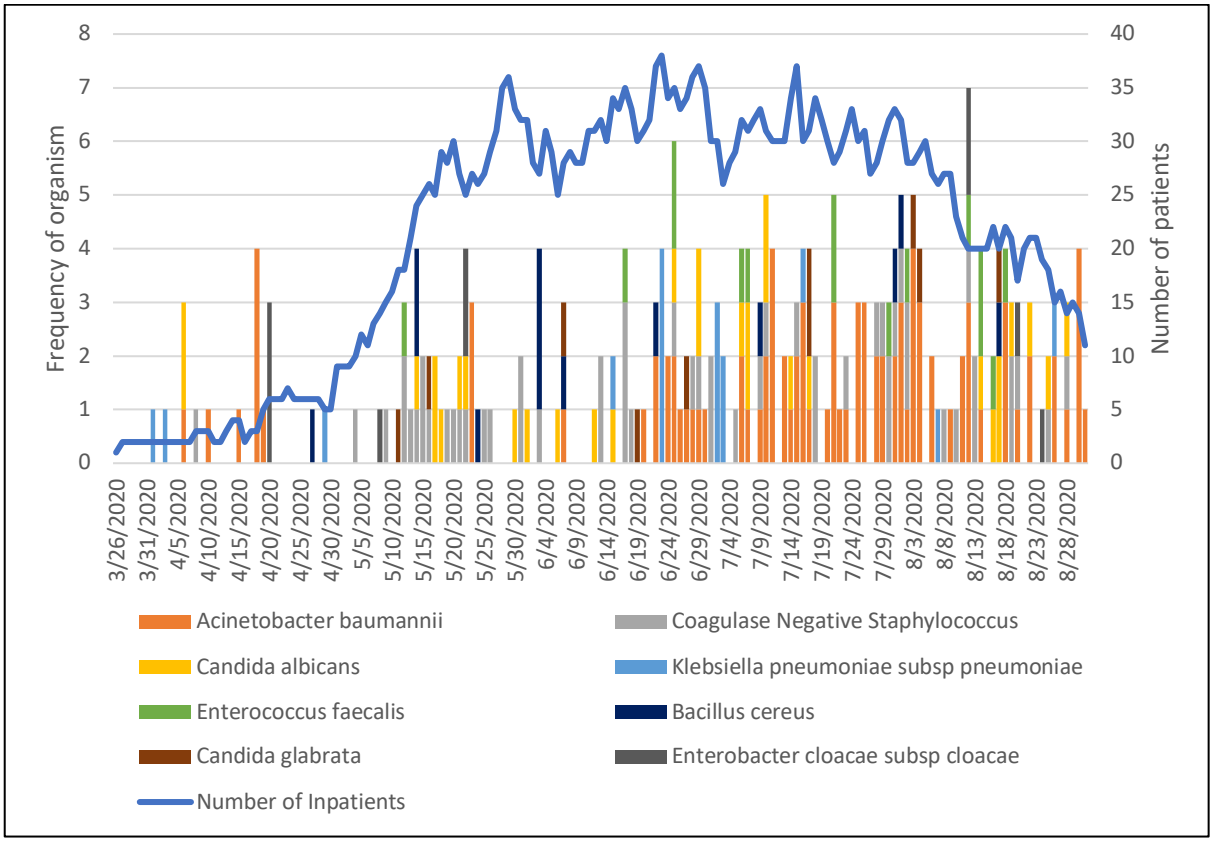

Figure S1a: Raw frequency of the most commonly identified organisms at all culture sites, prior to assessment for possible contamination and removal of duplicates, during the course of the pandemic.

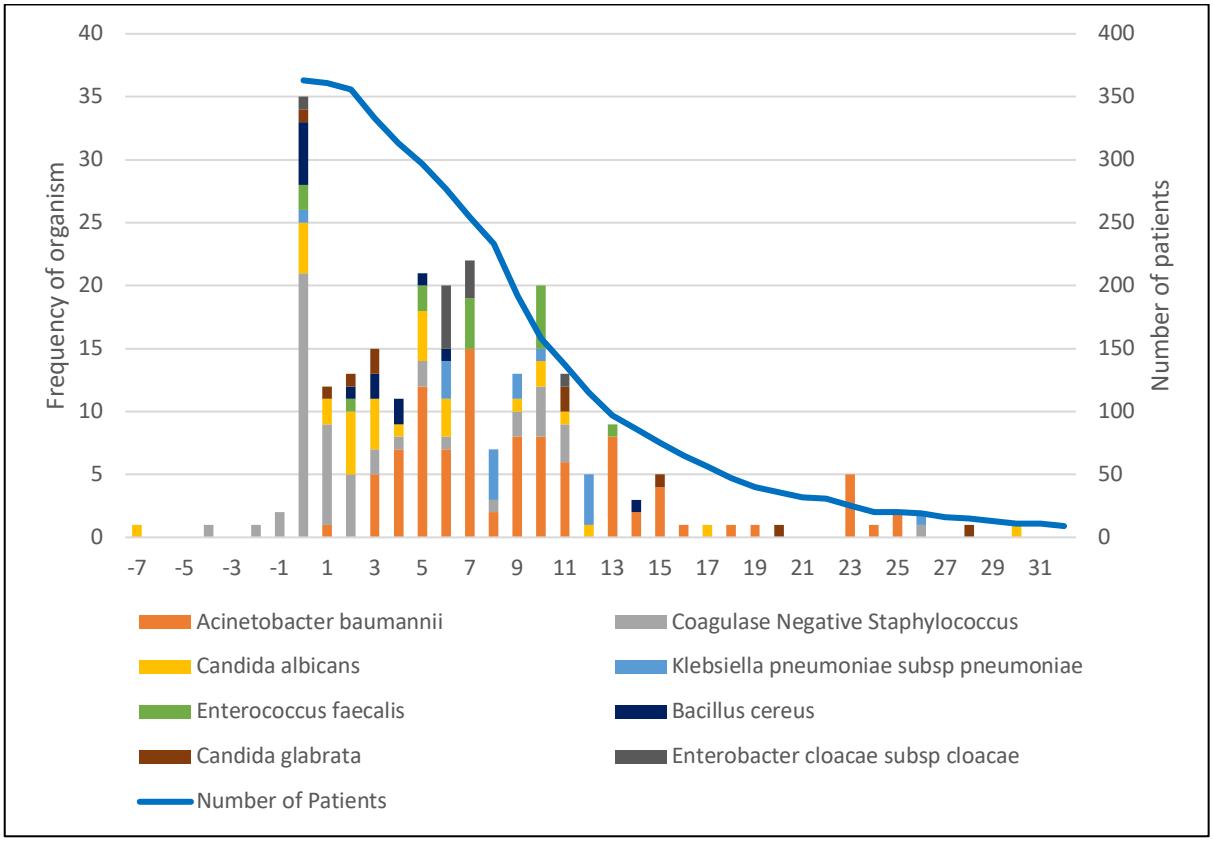

Figure S1b: Raw frequency of the most commonly identified organisms at all culture sites, prior to assessment for possible contamination and removal of duplicates, according to the patient day in the intensive care unit when the sample was obtained.

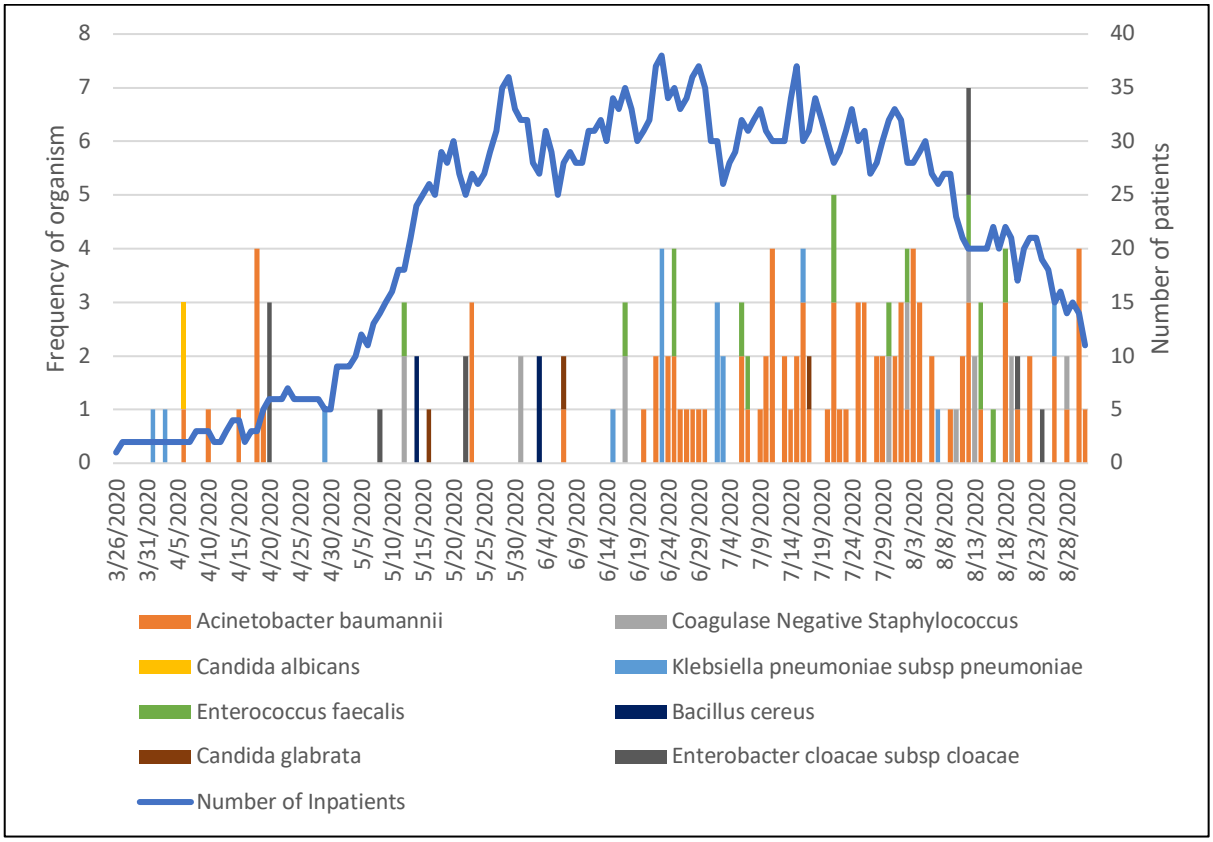

Figure S2a: Frequency of the most commonly identified pathogens at all culture sites, prior to removal of duplicates, during the course of the pandemic.

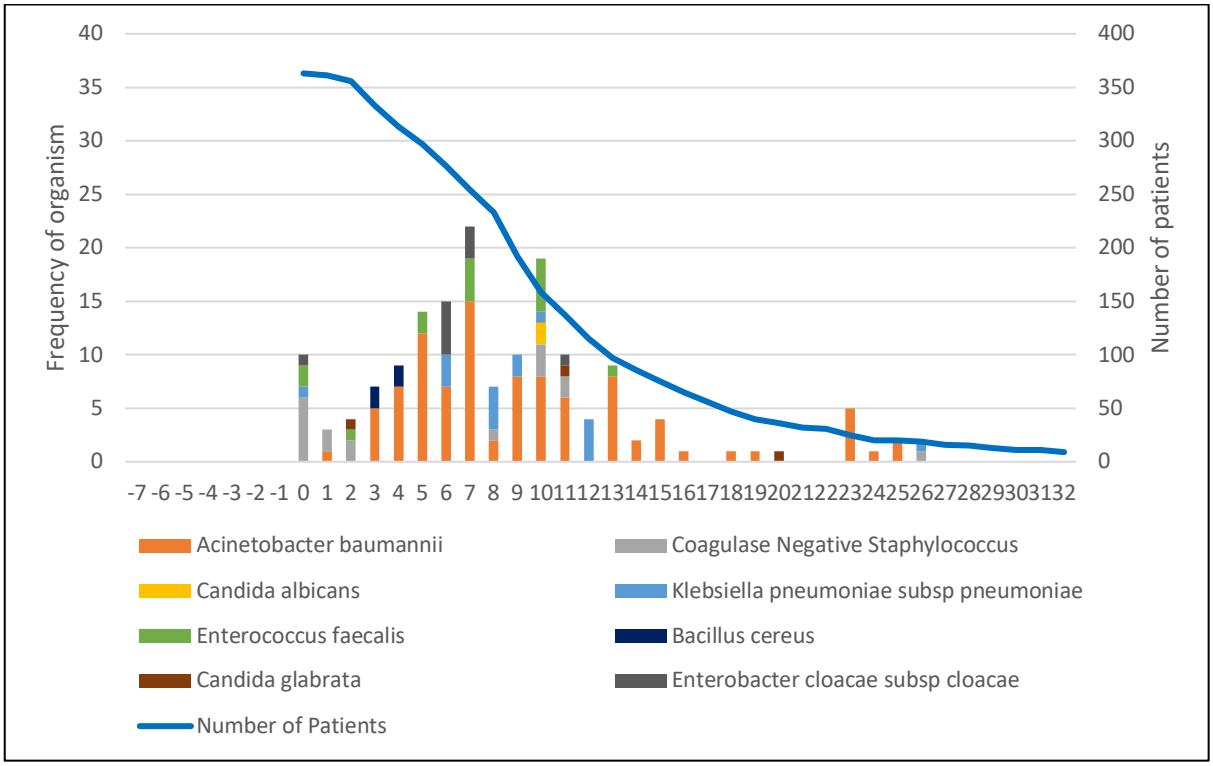

Figure S2b: Frequency of the most commonly identified pathogens at all culture sites, prior to removal of duplicates, according to the patient day in the intensive care unit when the sample was obtained.

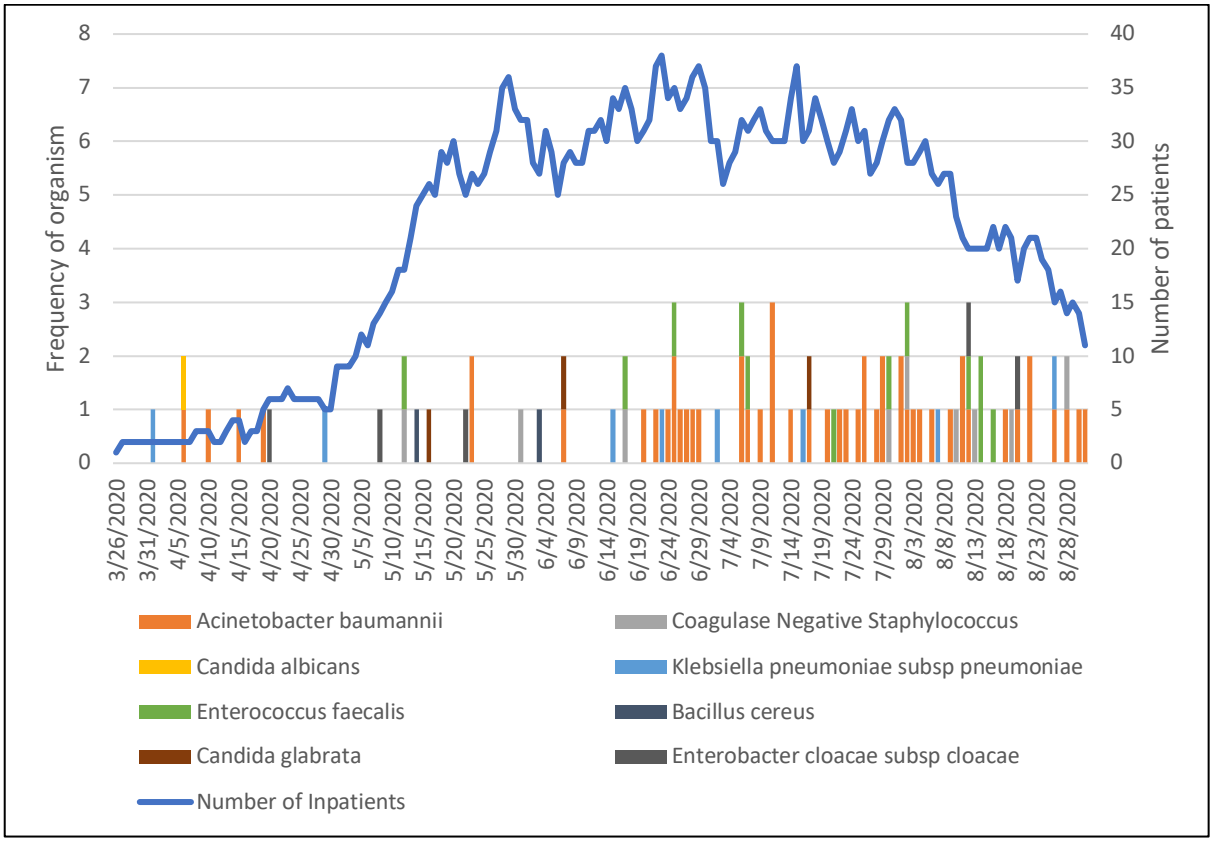

Figure S3a: Final frequency of the most commonly identified pathogens at all culture sites, after exclusion of duplicates, during the course of the pandemic.

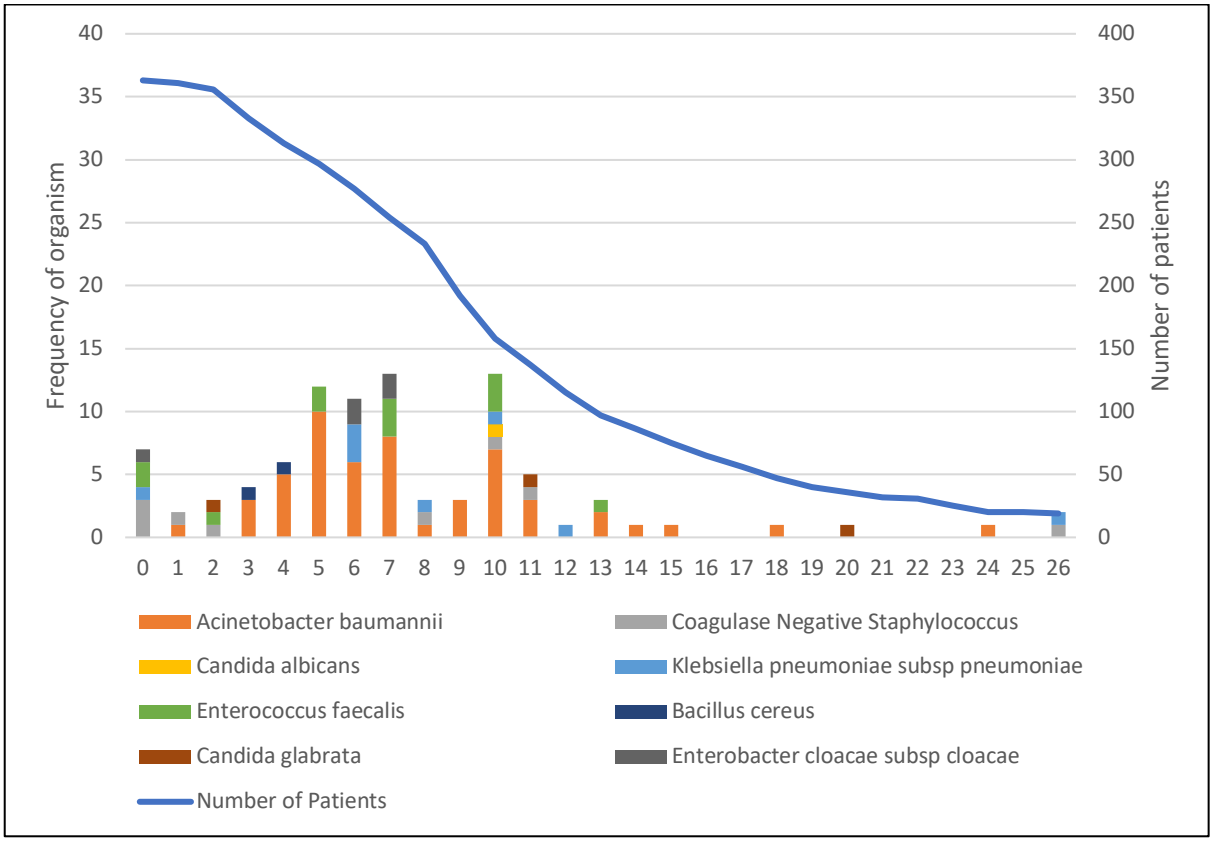

Figure S3b: Final frequency of the most commonly identified pathogens at all culture sites, after exclusion of duplicates, according to the patient day in the intensive care unit when the sample was obtained.

We subsequently assessed cultures to likely represent infection or contamination/colonisation according to the following rules:

Candida species including C. albicans, C. glabrata, C. tropicalis and C. parapsilosis, together with other yeasts were considered contaminants (or colonisers) if grown on tracheal aspirate or urine cultures and pathogens if detected on blood culture.

Staphylococcal cultures, including coagulase-negative staphylococci, *S. epidermidis, S. hominis* and *S. saccharolyticus*, together with *Bacillus* species, *Corynebacterium* species, *Micrococcus* species were considered contaminants (or colonisers) if grown on one culture only, and infection if grown on two or more consecutive cultures.

Environmental non-fermenters, including *Chryseobacterium indologenes* and *Pseudomonas fluorescens* were considered contaminants (or colonisers) if grown on tracheal aspirate or urine cultures and infection if detected on blood culture. *Aspergillus* and *Clostridium* species were classified based on the clinical setting. Other organisms were all considered pathogens.

Figure S2 shows all occurrences of the most common organisms after contaminants were excluded.

The rules according to which we removed duplicates are described in the main manuscript. Figure S3 shows the frequency of the most common organisms after both contaminants and duplicates were excluded.

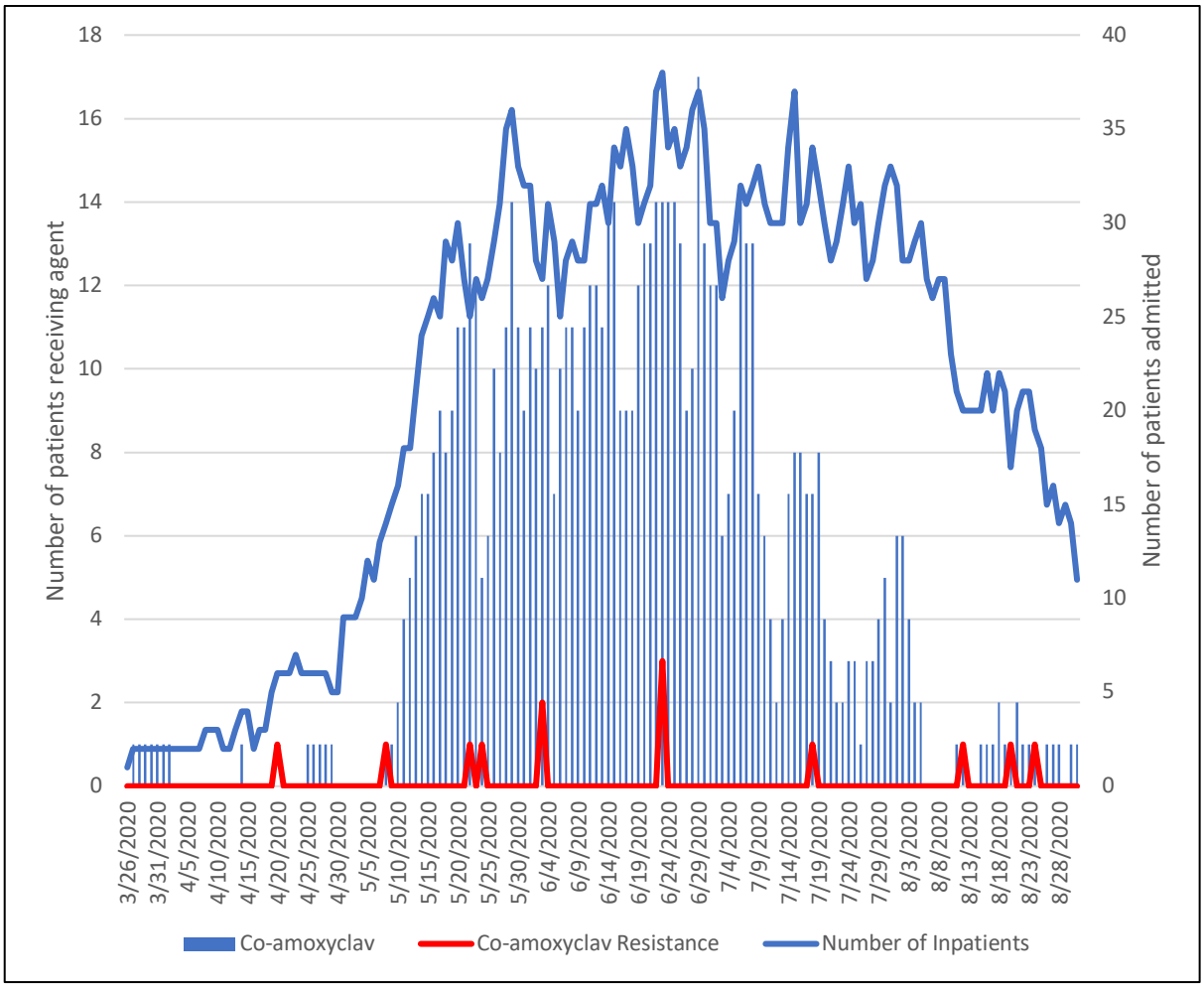

Figure S4a: Number of patients receiving amoxicillin-clavulanate by day during the course of the pandemic and instances of antimicrobial resistance to this agent.

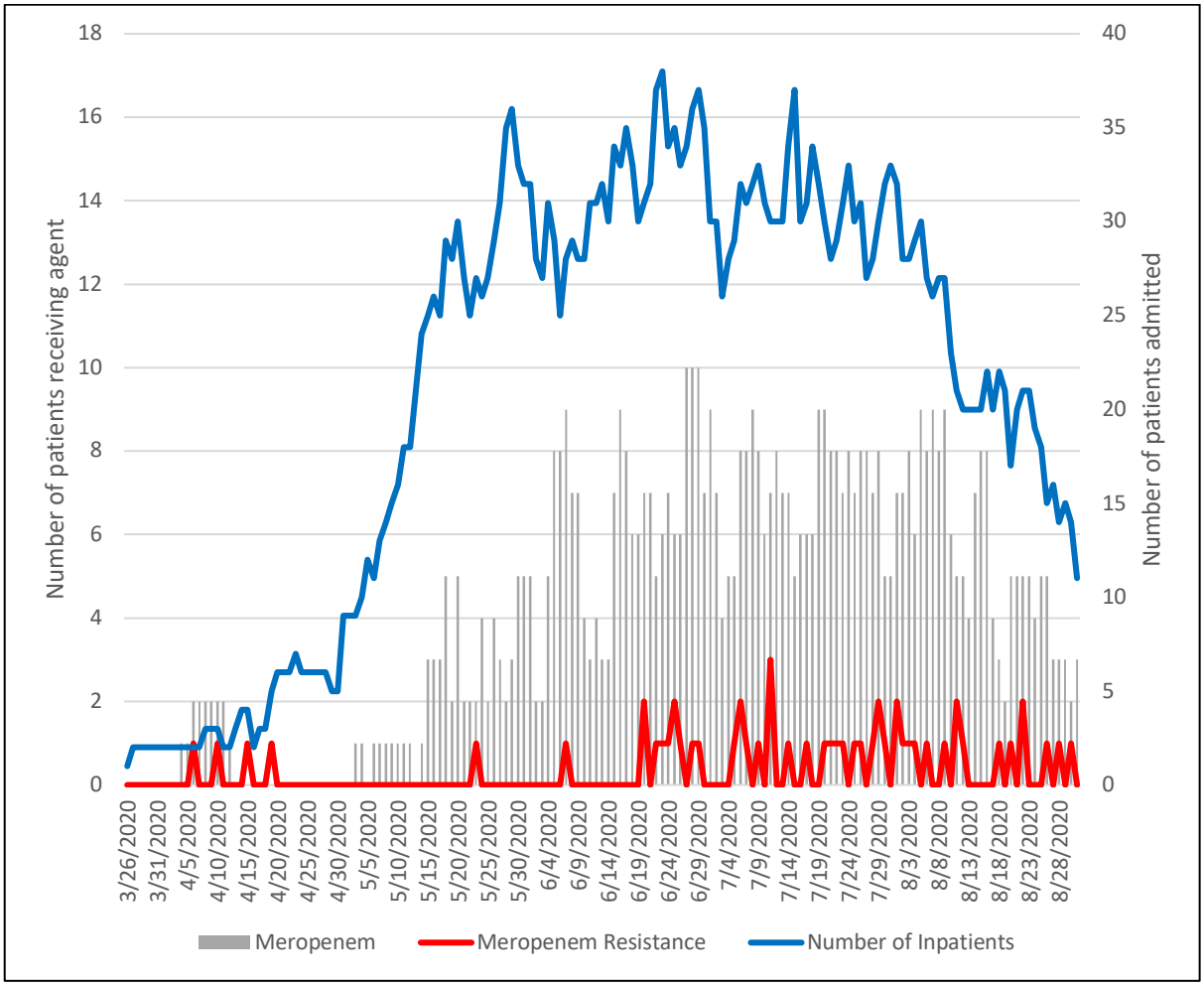

Figure S4b: Number of patients receiving meropenem by day during the course of the pandemic and instances of antimicrobial resistance to this agent.

Antimicrobial Use

For each day of the pandemic, we counted the number of patients receiving various antibiotics. Figure 1 (manuscript) shows the use of various antibiotics by day during the study period. Amoxicillin-clavulanate, azithromycin and meropenem were predominantly used, in keeping with unit protocols.

Figures S4 shows the use of two of these antibiotics with a comparison to all de-duplicated instances of resistance to these antibiotics. The graph depicts a clear increase in the occurrence of meropenem resistance in the latter half of the study period, following sustained use of the agent. However, the short duration of the study and an outbreak of *Acinetobacter baumannii* during the period in question made statistical analysis of this finding impossible.

The lack of a similar finding for amoxicillin-clavulanate may be due to testing practices at our local laboratory.