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Multidrug resistance assessment of indoor air in Portuguese long-term and acute healthcare settings

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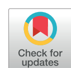

SUMMARY

Background: Knowledge about air as a pool of pathogens and multidrug resistance (MDR) in healthcare units apart from hospitals is scarce.
Aim: To investigate these features in a Portuguese long-term healthcare unit (LTHU) and a central hospital (CH).
Methods: Air samples were collected and their microbial load (bacteria and fungi) determined. Bacterial isolates were randomly selected for further characterization, particularly identification by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, antimicrobial susceptibility testing, and polymerase chain reaction screening of extended-spectrum β-lactamases, carbapenemase genes and *mecA* gene, with RAPD profile assessment of positive results of the latter.
Findings: A total of 192 samples were collected (LTHU: 86; CH: 106). LTHU showed a statistically significantly higher bacterial load. CH bacteria and fungi loads in inpatient sites were statistically significantly lower than in outpatients or non-patient sites. A total of 164 bacterial isolates were identified (MALDI-TOF: 78; presumptively: 86), the majority belonging to *Staphylococcus* genus (LTHU: 42; CH: 57). The highest antimicrobial resistance rate was to erythromycin and vancomycin the least, in both settings. Eighteen isolates (11%) were classified as MDR (LTHU: 9; CH: 9), with 7 MDR *Staphylococcus* isolates (LTHU: 4; CH: 3) presenting *mecA*. Nine non-MDR *Staphylococcus* (LTHU: 5; CH: 4) also presented *mecA*.
Conclusion: The current study highlights that healthcare unit indoor air can be an important pool of MDR pathogens and antimicrobial resistance genes. Also, LTHUs appear to have poorer air quality than hospitals, as well as supportive areas compared to curative care areas. This may suggest possible yet unknown routes of infection that need to be explored.

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Introduction

Healthcare-associated infections (HAIs) are infections related to direct healthcare, occurring after 48 h of hospitalization or within 30 days after discharge. On average, one in every 14 patients acquires an HAI [1]. In 2050, the number of antimicrobial resistance (AMR)-related infections will lead to 10 million deaths/year, more than the mortality due to cancer or diabetes [2]. HAIs caused by multidrug-resistant (MDR) organisms are even more serious as these infections are more difficult to treat, due to their ability to concomitantly resist the action of multiple antimicrobials [2,3]. Infection prevention and control (IPC) measures are practical, evidence-based approaches designed to halt pathogens and AMR/MDR transmission, maximally reduce HAI incidence, and protect patients and healthcare workers (HCWs) [4–7].

A healthcare facility environment poses as a major reservoir of pathogens, including bacteria, fungi and viruses [8,9]. Great attention is already placed on hand hygiene, respiratory etiquette and surface disinfection, but little attention is dedicated to air quality, also considered important for patient and HCW biosafety and health [10,11]. Poor indoor air quality in healthcare settings has already been linked to increased HAI and work-related respiratory diseases [11,12]. Recent evidence demonstrates a link between particulate matter air pollution and increased AMR; however, literature remains scarce regarding concerning AMR and microbial diversity and distribution in healthcare air [13]. Moreover, available literature focuses mainly on hospitals, neglecting long-term healthcare units (LTHUs), which are increasingly accommodating more elderly patients with higher levels of frailty and longer histories of antimicrobial consumption [11,14]. Also, there is already some evidence that LTHUs may have higher loads of MDR pathogens and AMR genes in their environment, but without recording their microbial air loads [15].

Quantifying and identifying air-borne microbes, assessing their AMR pool, and interpreting which possible factors may contribute to AMR dissemination, are important to deliver knowledge that can guide better IPC programmes in these healthcare settings. The present study aim was to describe indoor air microbial quality in an LTHU and a central hospital (CH), including their AMR features and which factors may influence microbial air quality.

Methods

LTHU and CH were sampled fortnightly for nine months, one sample per site in each sampling day. LTHU sampling was focused on patient rooms (PR), supportive healthcare rooms (SR) and general activity rooms (GR). CH sampling focused on four care provision areas: intensive care unit (ICU), emergency department (ED), intermediate care unit (IMCU), pulmonology outpatient clinic (POC); and three supportive services areas: cafeteria (CAF), microbiology laboratory (LAB), and sterilization unit (STERIL).

Sampled sites were characterized according to area (m^2), room capacity (average person/room), patient occupation (inpatient, outpatient, non-patient), type of activity (according to System of Health Accounts of healthcare functions [16]), and their potential location-related infection risk (following Table 2 of infection control risk assessment 2.0 tool) [17].

Air samples were collected using an air sampler (MAS-100 VF®, Merck Millipore), according to the manufacturer's instructions. Bacterial quantification was performed using plate count agar (PCA) (Alliance Bio Expertise, Bruz, France), 30 °C, three days, and fungal quantification using Rose Bengal agar (RBA) (Alliance Bio Expertise, Bruz, France), 25 °C, five days [18]. Number of colony-forming units (cfu) was recorded (cfu/m^3). Bacterial isolates were further characterized. Fungal isolates were only used for the quantitative study.

From PCA plates with bacterial growth, two colonies per plate were randomly selected for further characterization. Each isolate was purified and cryopreserved at −80 °C. Randomly selected isolates were identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry [19]. Resulting MALDI-TOF fingerprints were interpreted using SARAMIS (AnagnosTec, Potsdam-Golm, Germany) and PAPMID (putative assigned protein masses for identification database; Mabritec, Riehen, Switzerland) databases [20]. Antimicrobial susceptibility testing (AST) and interpretation was performed following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [21]. To increase overall AMR information, since it was not possible to identify all preserved isolates, presumptive identifications were made based on phenotypic similarities with those identified, to allow interpretation of non-identified isolates' AST profiles. *mecA* gene was polymerase chain reaction (PCR)-screened in all MALDI-TOF-identified or presumptively identified *Staphylococcus* isolates, using a 25 μL mixture of a commercially available solution of DNA polymerase, Mg^{2+}, dNTs, and dye (NZYTaq II 2× Green Master Mix; NZYtech, Lisbon, Portugal) with the target primers at 10 μM and 1 μL of the target DNA, per each sample. The reaction consisted of 30 cycles of amplification at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, and included an initial denaturation at 94 °C for 3 min and a final extension at 72 °C for 30 s [22]. Genotypic polymorphism was assessed by RAPD–PCR on isolates testing positive for the *mecA* gene [23].

Extended spectrum β-lactamase (ESBL) genes ($bla_{CTX-M-G1}$, $bla_{CTX-M-G2}$, $bla_{CTX-M-G8}$, $bla_{CTX-M-G9}$, $bla_{CTX-M-G25}$) were PCR-screened in all non-*Staphylococcus* isolates with synergy phenomena between amoxicillin with clavulanic acid (AMC) discs, cefotaxime (CTA) and ceftazidime (CTZ) discs in AST [24]. Carbapenemase genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{PKC}, bla_{OXA-48}) were PCR-screened in all non-*Staphylococcus* isolates with phenotypic resistance to imipenem [25]. Similar PCR conditions to those described above, with adjusted annealing temperatures, were used in this screen.

Statistically significant association between bacterial or fungal cfu/m^3 and air-sampled site variables were sought for both units, using IBM SPSS® version 28. For all analysed

variables the significance level was defined as $P < 0.05$. Since data did not show a normal distribution, only non-parametric tests were used, followed by post-hoc tests to determine which variables contributed to the detected statistically significant differences. Regressions were made with Microsoft Excel 2016® to show bacterial and fungal load trends according to area and room capacity.

Results

A total of 192 air samples were collected, 86 from LTHU and 106 from CH (Table I). Mean area and room capacity of sampled sites at LTHU was six and two times smaller than in CH (20.6 ± 8.6 vs 124.2 ± 70.8 m^2; 4 ± 5 vs 18 ± 15 persons/room), respectively. The majority of LTHU samples were collected in inpatient rooms, while a more homogeneous sample distribution per sites was conducted in CH. As to location-related infection risk, LTHU samples were obtained in sites classified as high risk (PR and GR, where inpatients circulate, receive therapy and perform several activities) and medium risk (SR, where only HCWs circulate), while CH samples were collected in sites classified as medium risk (CAF), high risk (ED, POC, IMCU, LAB) and highest risk (ICU, STERIL) (Table I).

Of the 192 air samples analysed, bacterial growth was observed in 125 samples (65.1%), while fungal growth was detected in 161 samples (83.9%). Mean bacterial load in LTHU was 367 cfu/m^3 (median: 222; SD: 381; range: 20–2290) and 230 cfu/m^3 (median: 170; SD: 215; range: 20–1010) in CH. Mean fungal load in LTHU was 87 cfu/m^3 (median: 30; SD: 144; range: 0–840) and 117 cfu/m^3 (median: 60; SD: 161; range: 0–860) in CH. Overall bacterial and fungal load distribution, variability and skewness in LTHU and CH sampled sites are presented in Figure 1. LTHU bacterial load was statistically significantly higher than in CH ($U = 3007.0$, $P = 0.006$) but not fungal load ($U = 4750.5$; $P = 0.052$). No statistically significant associations between LTHU room, area, patient occupation, type of activity and location-related infection risk and their bacterial loads were observed.

However, bacterial load in CH showed statistically significant differences between the different sampled sites ($H(6) = 25.133$, $P < 0.0005$), patient occupation ($H(2) = 8.538$, $P = 0.014$), type of activity ($H(3) = 16.132$, $P = 0.001$) and location-related infection risk ($U = 812.0$, $P = 0.032$). Post-hoc tests revealed that median bacterial load in CAF (median: 78.14) was significantly higher than in ICU (median: 36.93) and LAB (median: 33.29), as well as in outpatient rooms (median: 63.42) compared to inpatient rooms (median: 40.50), supportive services (median: 78.14) compared to curative-care rooms, medical goods and ancillary services rooms (median: 52.08, 48.20, 33.29, respectively). Regarding location-related infection risk, median bacterial cfu/m^3 in high-risk rooms was significantly higher than in highest risk rooms (median: 56.53 vs 42.57, respectively).

Considering fungal loads, no statistically significant differences were observed within LTHU variables. As to CH, statistically significant differences were observed between sample sites ($H(6) = 41.797$, $P < 0.0005$), patient occupation ($H(2) = 20.762$, $P < 0.0005$) and type of activity ($H(3) = 17.900$, $P < 0.0005$), but not regarding location-related infection risk. Post-hoc tests showed that median fungal load in IMCU (median: 22.21) was lower than in CAF, STERIL, POC and LAB (median: 75.03, 70.81, 76.32, 64.28 respectively), and also ED (median: 40.88) compared to CAF and POC (median: 75.03 and 76.32); as well as in inpatient rooms (median: 32.00) compared to outpatient and non-patient rooms (median: 51.43 and median: 64.53), and curative-care rooms (median: 41.56) compared to support services and medical goods rooms (median: 71.68 and 65.77).

Table I

Characterization and classification of long-term healthcare unit (LTHU) and central hospital (CH) sampled sites and respective number of collected air samples

Variable	LTHU ($N = 86$)	CH ($N = 106$)
Sample site	PR: $n = 53$ SR: $n = 17$ GR: $n = 16$	STERIL: $n = 15$ ED: $n = 15$ ICU: $n = 15$ CAF: $n = 14$ POC: $n = 16$ IMCU: $n = 16$ LAB: $n = 15$
Area (m^2)	Mean: 20.6 (SD: 8.6; range: 5.1–44.7)	Mean: 124.1 (SD: 70.8; range: 25.0–232.0)
Capacity (persons/room)	Mean: 4 (SD: 5; range: 1–20)	Mean: 18 (SD: 14; range: 2–50)
Patient occupation	Inpatient: $n = 70$ No patient: $n = 16$	Inpatient: $n = 29$ Outpatient: $n = 31$ No patient: $n = 46$
Type of activity	Curative care: $n = 6$ Long-term care: $n = 71$ Support services: $n = 9$	Curative care: $n = 62$ Ancillary services: $n = 15$ Medical goods: $n = 15$ Support services: $n = 14$
Location-related infection risk	Medium risk: $n = 9$ High risk: $n = 77$	Medium risk: $n = 14$ High risk: $n = 62$ Highest risk: $n = 30$

CAF, cafeteria; ED, emergency department; GR, general activities rooms; ICU, intensive care unit; IMCU, intermediate care unit; LAB, laboratory microbiology room; PR, patient room; POC, pulmonology outpatient clinic; SR, support healthcare room; STERIL, sterilization unit; SD, standard deviation.

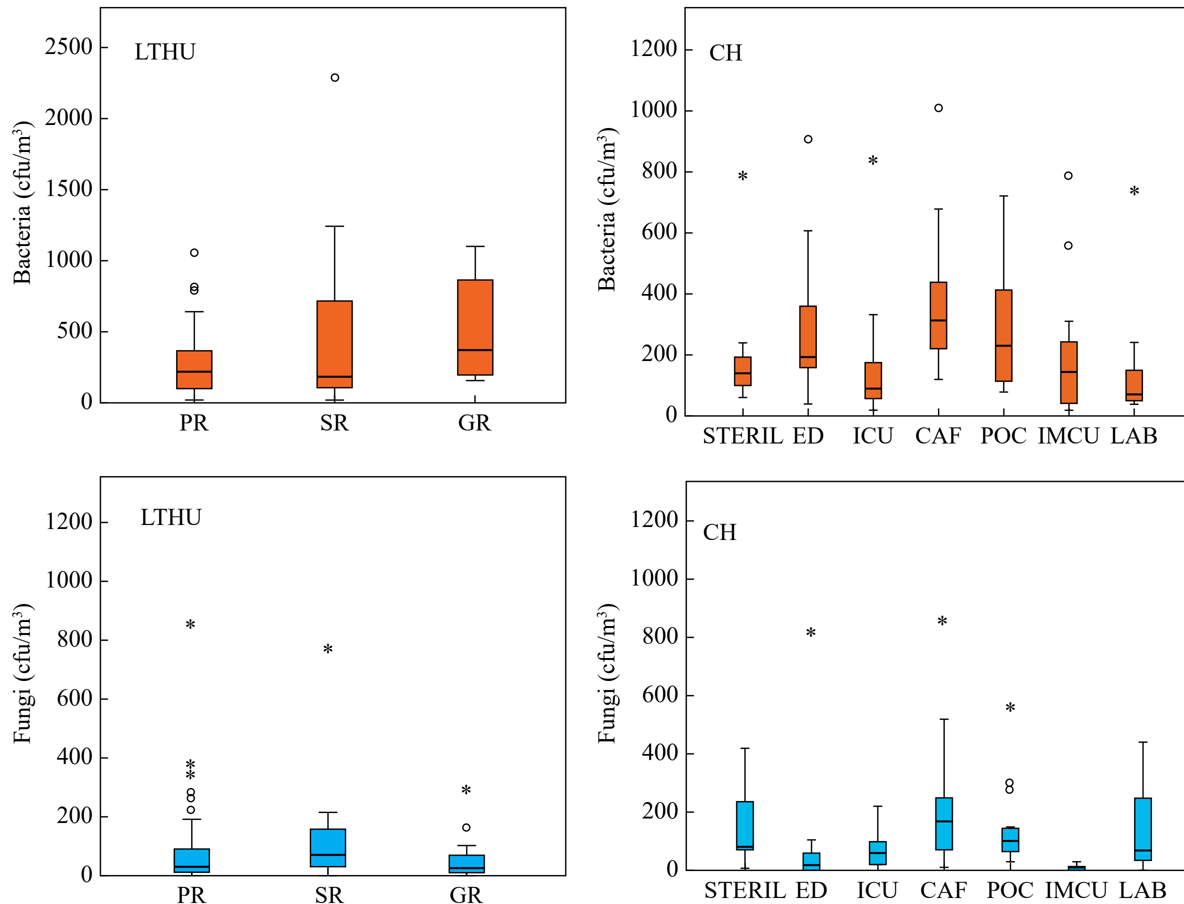

Figure 1. Comparative distribution of air-borne bacterial and fungal load (cfu/m^3) in long-term healthcare unit (LTHU) (left side) and central hospital (CH) (right side) sampled sites. Centre lines show the medians, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by dots and stars. Note that LTHU bacterial load graphic has a different *y*-axis scale, due to the higher observed cfu/m^3. CAF, cafeteria; ED, emergency department; GR, general activities rooms; ICU, intensive care unit; IMCU, intermediate care unit; LAB, laboratory microbiology room; PR, patient room; POC, pulmonology outpatient clinic; SR, support healthcare room; STERIL, sterilization unit.

Linear regressions were made to study bacterial and fungal load trends in LTHU and CH according to room area and capacity. However, low R-values were obtained, so the models are not suitable for predictions. Bacterial cfu/m^3 increased with room area in both settings and with room capacity in CH, but not in LTHU, where higher occupancy correlated with lower airborne bacteria levels. Fungal load trends showed an opposite pattern, decreasing with larger room area and capacity in LTHU, while decreasing with larger room area but increasing with higher occupancy in CH.

After performing this quantitative analysis, we were interested in analysing the quality of the air-borne bacteria in both settings, as well as their AMR phenotypic and genotypic pool. No qualitative study was conducted regarding the fungal samples. A total of 164 bacterial isolates were randomly preserved, 67 from LTHU (PR: 36; SR: 13; GR: 18), 97 from CH (ED: 21; ICU: 14; CAF: 11; POC: 22; IMCU: 13; LAB: 6; STERIL: 10), with 38 LTHU and 40 CH isolates being MALDI-TOF identified. The remaining 29 LTHU and 57 CH isolates were presumptively identified by comparison with those already identified, according to their phenotypic similarities and epidemiological contexts. More than half of LTHU isolates were identified as *Staphylococcus* spp. (MALDI-TOF: 23; presumptive: 19). From the 25 non-*Staphylococcus* LTHU isolates, *Corynebacterium* spp. (8), *Pseudomonas* spp. (2), *Pantoea* spp. (2), *Roseomonas mucosa* (1), *Bacillus pumilus* (1), *Serratia liquefaciens* (1), and 10 presumptive Enterobacterales were also identified.

As for CH isolates, the majority were also identified as *Staphylococcus* (MALDI-TOF: 16; presumptive: 41). From the remaining 40 non-*Staphylococcus* CH isolates, *Corynebacterium* spp. (11), *Acinetobacter* (3), *Pseudomonas* spp. (3), *Roseomonas* spp. (2), *Bacillus cereus* (1), *Burkholderia pseudomallei* (1), *Brevundimonas vesicularis* (1), *Moraxella osloensis* (1), *Pantoea brenneri* (1), and 16 presumptive Enterobacterales were also identified.

Globally, *Staphylococcus* isolates presented high AMR rates to erythromycin (ERY) (39), norfloxacin (NOR) (23) and tetracycline (TET) (20). As to non-*Staphylococcus* isolates, aztreonam (AZT) (26) was the antimicrobial with the highest AMR rate (Figure 2). In LTHU, *Staphylococcus* presented higher AMR rates to NOR and ERY (15, both), and non-*Staphylococcus* to AZT (11) and clindamycin (CLI) (7). In CH, the majority of *Staphylococcus* were resistant to ERY (24) and TET (10), and non-*Staphylococcus* to AZT (20), CTZ and cefiderocol (CFD) (10, both). According to MDR definition, the acquired non-susceptibility to at least one agent from three or more antimicrobial categories, 18 (11%)

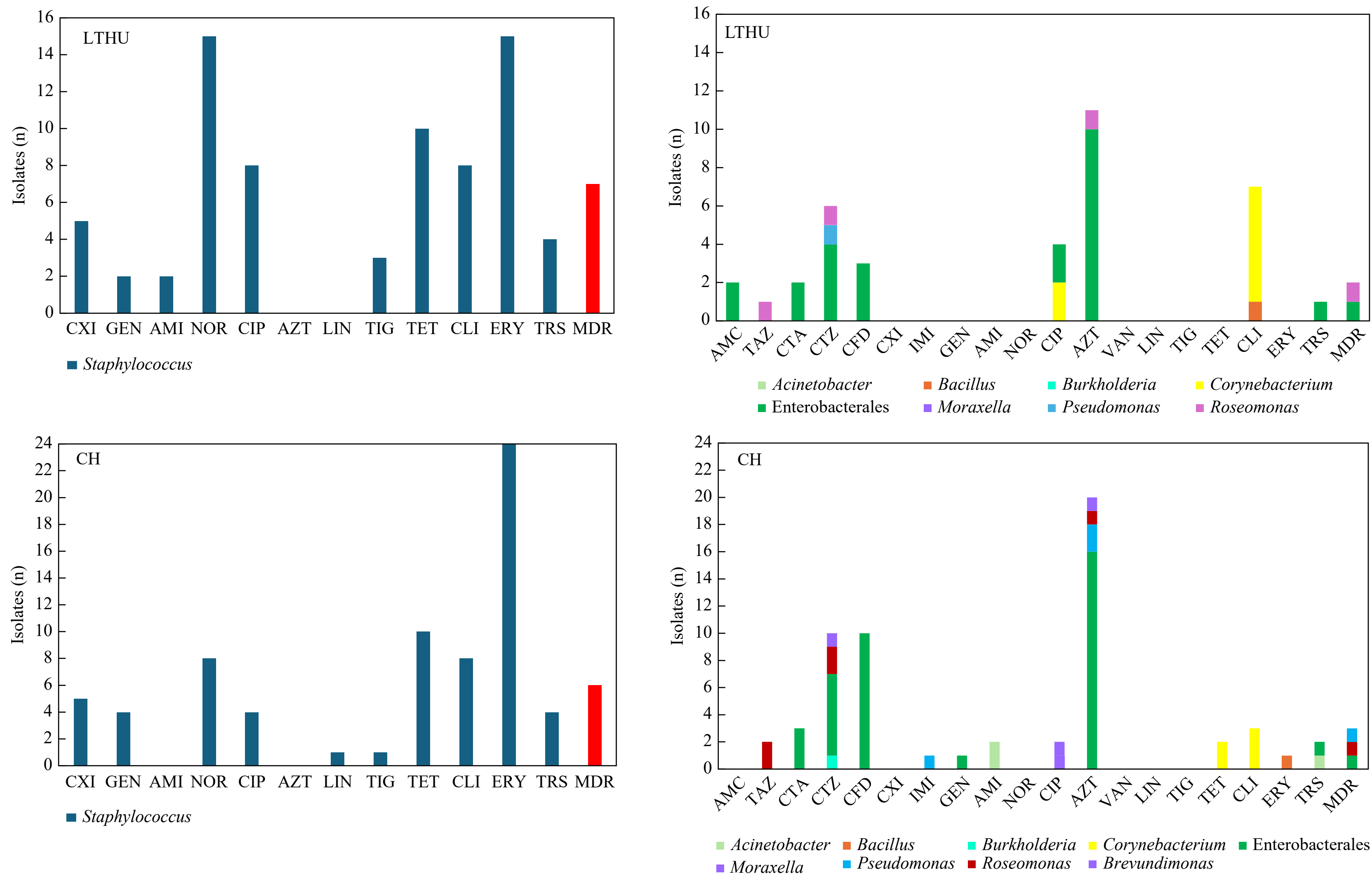

Figure 2. Long-term healthcare unit (LTHU) and central hospital (CH) overall antimicrobial resistance. AMC, amoxicillin with clavulanic acid; AMI, amikacin; AZT, aztreonam; CFD, cefiderocol; CIP, ciprofloxacin; CLI, clindamycin; CTA, cefotaxime; CTZ, ceftazidime; CXI, cefoxitin; ERY, erythromycin; GEN, gentamicin; LIN, linezolid; IMI, imipenem; MDR, multidrug resistant; NOR, norfloxacin; TAZ, tazobactam; TET, tetracycline; TIG, tigecycline; TRS, trimethroprim—sulfamethoxazole; VAN, vancomycin.

were classified as MDR (LTHU: 9; CH: 9) (Figure 2): 13 *Staphylococcus* spp. (LTHU: 7; CH: 6), 2 *R. mucosa* (LTHU: 1; CH: 1), 2 presumptive Enterobacterales (LTHU: 1; CH: 1), and 1 *B. vesicularis* (CH: 1) (Figure 2).

The LTHU MDR isolates were all collected from PR, 2 *S. haemolyticus* (PR10 and 17), 2 *S. aureus* (PR10 and PR15), 2 *S. hominis* (PR5 and PR20), 1 *R. mucosa* (PR8) and 1 presumptively identified Enterobacterales (PR8), except 1 *S. haemolyticus*, collected in the dining hall (GR). Of note, *R. mucosa* and Enterobacterales MDR isolates were collected from the same room (PR8), in the same day, with different phenotypical AST profiles. The *R. mucosa* isolate exhibited resistance to penicillins, cephalosporins, and monobactams, while the Enterobacterales isolate showed resistance to the same antimicrobial classes plus the miscellaneous agents. Both MDR *S. aureus* were collected in the same day, but from different rooms, with 1 being collected from the same room as *S. haemolyticus* (PR10).

In CH, most MDR isolates were *Staphylococcus*, 3 *S. haemolyticus* (IMCU: 2; ICU: 1), 2 *S. hominis* (IMCU: 1; POC: 1), 1 *S. saprophyticus* (POC), 1 *R. mucosa* (ICU), 1 *B. vesicularis* (ICU), and 1 presumptively identified Enterobacterales (ED). There were no similarities in species or AST profile in MDR isolates collected in CH rooms under study, over time, except 2 *S. haemolyticus*, that were both collected from IMCU in a two-month interval and shared a similar phenotypic AST profile (both isolates showed resistance to cefoxitin, gentamicin, NOR ciprofloxacin, TET, ERY and trimethoprim–sulfamethoxazole).

Regarding AMR genes (ARG), 16 *Staphylococcus* isolates presented *mecA*, 7 classified as MDR (LTHU: 4; CH: 3) and 9 as non-MDR (LTHU: 5; CH: 4). The MDR isolates positive for *mecA* were 4 *S. hominis* (LTHU: 2; CH: 2) and 3 *S. haemolyticus* (LTHU: 2; CH: 1). The non-MDR *Staphylococcus* with *mecA* were 2 *S. cohnii* (LTHU), 1 *S. caprae* (LTHU) and 4 presumptive *Staphylococcus* (LTHU: 1; CH: 3). In LTHU, *mecA* was detected in 5 different PR, 2 SR (both in treatment room, 6 months apart) and 2 GR (both in dining hall, 5 months apart). In CH, *mecA* was detected in ICU (2 isolates, 4 months interval), POC and IMCU (2 isolates each, 2 months apart), and ED (1 isolate). No ESBL or carbapenemase genes were detected in any of the tested non-*Staphylococcus* isolates. RAPD analysis of *Staphylococcus* isolates (MALDI-TOF identified and presumptive) positive for the *mecA* gene revealed different profiles within this group.

Discussion

In healthcare settings, it is irrefutable that some HAIs are air-borne, with pathogen transmission occurring via aerosol and droplets caused by patients, HCWs or visitors. However, little is known about which pathogens persist in the air, nor about their AMR pool [13]. This study aimed to characterize indoor air-borne bacterial and fungal load from different healthcare settings, assess bacterial AMR pool and determine possible associations to environmental and organizational factors, such as room dimension and capacity, type of activity, inpatient presence and infection risk, to determine whether they may influence the persistence of pathogens and AMR in the air of long-term and acute healthcare settings.

A systematic review regarding indoor air quality in healthcare units reported that 91% of overall studies are performed in hospitals, highlighting the relevance of studying other healthcare units [11]. The present comparative study between LTHU and CH, where statistically significantly higher bacterial loads in LTHU were observed, further corroborates it. Although the current study gathered no information on outdoor air, meteorological and seasonal factors, building structures and ventilation systems, these factors all may impact indoor air quality [26–29]. Further studies should explore these possibilities in order to better understand the differences between LTHU and hospital indoor microbial air quality.

No international standardization on indoor air microbial quality is available for healthcare settings. Every country follows its own standards, if available, adapting existing standards designed for different settings to healthcare facilities. This results in a wide range of different values for acceptable maximum bacterial and fungal loads in healthcare indoor air in different countries; such values may not be adequate for healthcare purposes, as these facilities typically serve an immunocompromised population, different from those from other settings [12]. Regarding Portugal, only Ordinance n. 353-A/2013 – encompassing commercial and services buildings, and afterwards ratified in 2021, then mentioning day-care centres, elementary schools and nursing homes – establishes that indoor air bacterial load should be lower than outdoor bacterial load plus 350, and fungal indoor air load lower than outdoor [30,31]. Since no outdoor air bacterial or fungal loads were obtained in the current study, it was not possible to interpret obtained results according to this legislation.

In the current study, only CH presented statistically significant differences between bacterial or fungal loads and the studied environmental factors. However, this might be due to the low number of samples/site in LTHU, due to the convenience approach of sampling in this setting compared to the purposive sampling approach in CH. In future studies, this problem should be circumvented.

In CH, bacterial and fungal loads were statistically higher in outpatients or non-patient sites, and in supportive services areas. Medium infection risk sites presented higher bacterial loads, but not fungal. These results partially agree with a study on hospital microbial air quality that suggests that bacterial and fungal bioaerosol concentrations are higher in inpatient and public areas compared to rooms with restricted access and/or requiring protective equipment (surgical rooms, ICU or oncology wards) [32]. Moreover, more powerful forced-air ventilation systems to secure higher air renovation in ICU is already demonstrated to provide better biosafety to ICU patients, staff, and visitors, by reducing the spread of airborne infections [33]. The current results corroborate this.

According to MALDI-TOF identification, most bacterial isolates were identified as *Staphylococcus*, but some Gram-negatives were also identified in both study settings. This concurs with a study conducted in a Portuguese hospital, where the predominantly identified genera were *Staphylococcus* (51%) and *Micrococcus* (37%), and Gram-negatives were also identified, including *Neisseria* (4%), *Proteus* (2%), and *Shigella* (0.3%) [34]. It is relevant to highlight the similarity between the air-borne species diversity and the human skin microbiota [35]. In healthcare settings, this may concur with a higher level of skin exposure that could facilitate the translocation of skin bacteria to the air. However, studies from different settings also indicate high levels of *Staphylococcus* in the air [36,37]. Future comparative studies could demonstrate whether

healthcare setting air microbiota is closer to human skin microbiota than in other settings.

Considering the scarce number of studies addressing AMR in healthcare air, we scoped the selected isolates' AMR features [38]. In both settings, isolates showed higher resistance rate to ERY. A recent study showed an increase in ERY resistance in meticillin-susceptible *S. aureus* (MSSA) from 13.6% in 2004 to 28.9% in 2020 ($P < 0.001$) in clinical isolates from hospital settings [39]. No literature was found regarding ERY resistance in air-borne pathogens.

Regarding MDR features, with 18 out of the total 164 (11%) LTHU and CH isolates classified as MDR, the current results are more encouraging than those obtained in an Ethiopian study, where, from 216 passive air samples, 75% isolates were considered MDR. Conversely, an Italian study that analysed MDR prevalence in the air of operating rooms and dental offices, showed that only 1% of indoor-air isolates presented MDR features [40]. These divergences help to corroborate the variability of microbial air quality in healthcare settings, and the importance of studying this problem to better address the potential pathogens and AMR/MDR dissemination through the air. In the current study, contrary to what was expected, MDR isolates incidence was equal between LTHU and CH, where the use of antimicrobials is much higher [41]. This demonstrates the rise of AMR and MDR in LTHUs, a relatively unknown problem that also needs to be rapidly addressed to prevent LTHUs becoming inadvertent pools of AMR and MDR for their inpatients and the community [42].

Airborne transmission of MDR pathogens and their ARG is significant in hospitals [15]. One study found 31 airborne ICU and operating room MRSA isolates having *mec*A [43]. Additionally, research comparing *mecA* and $bla_{CTX\text{-}M}$ gene abundance in five Chinese hospitals' bioaerosols revealed that *mec*A was more prevalent in overall units (transfusion area, outpatient and inpatient), while $bla_{CTX\text{-}M}$ was more abundant in inpatient areas [15]. Current results, in a similar way, showed that both in acute healthcare units (ICU, ER, and IMCU) and several rooms in LTHU, *mecA* was detected and persisted over time. Studies on clonality would be interesting to establish relationships between isolates. Finally, and although no ESBL nor carbapenemase genes were yet detected, if nothing is done to control the spread of MDR in LTHU, we may assist the rise of 'superbugs' also in this setting in the near future. It is imperative to update IPC programs to include measures to improve microbial air quality, to better protect patients, HCWs, and the general community [29,44].

The current study has some important limitations to point out, namely the difference in number of air samples per site between both healthcare units, information on forced or passive air ventilation and number of inpatients and other people in the room during sampling, as well as environmental information such as room temperature, humidity, and the inability to test outdoor microbial loads. In future studies these pitfalls should be addressed, potentially with significant contributions from a tool such as Hazard Analysis and Critical Control Points (HACCP). Developed in the 1960s to ensure safe food for space missions, HACCP has been widely adopted in food and pharmaceutical industries. Although its implementation in healthcare settings is less well documented, HACCP principles of process-orientation, in-process safety control, and hazard analysis can be applied to clinical context [45]. This approach could enhance IPC and address modern healthcare challenges, as AMR, MDR, and ARG spread [46].

The present study compared the microbial indoor air quality of Portuguese LTHU and CH, and their bacterial AMR pool. Considering overall demographic ageing and increment of chronic illness, with subsequent need of permanent long-term care to the elderly, LTHUs are becoming increasingly relevant in all countries' national healthcare systems [47,48]. Until very recently, IPC programmes were not generally recognized to be required in these settings, but increasing evidence, including the current study, highlights their mandatory need, without neglecting air microbial quality [49].

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Author contributions

C.S.-M.: validation, formal analysis, investigation, data curation, writing – original draft, and visualization. C.T.: investigation, data curation, and writing – original draft. R.P.: formal analysis and writing – original draft. W.M.B.: conceptualization, validation, resources, writing – review and editing, and funding acquisition. S.G.P.: conceptualization, methodology, validation, resources, writing – review and editing, supervision, project administration, and funding acquisition.

Conflict of interest statement

None to declare.

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Ethical approval

Not required.

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