

## Online data Supplement

Manuscript title: Microbial aetiology of Health Care Associated Pneumonia (HCAP) in Spain: a prospective, multicenter, case-control study.

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### **Definitio criteria for HCAP:**

Prior hospitalization ( $\geq 48$ h) in the last 2 months; b. NH Residence; c. Outpatient haemodialysis or infusion therapy; d. Domiciliary wound care; e. Close contact with MDR infection.

### **Viral microbiological investigation:**

Respiratory viruses were studied through a reverse transcriptase PCR technique (CLART PneumoVir, Genomica) consisting in 2 phases: 1. the *amplification* of a specific 120-330 bp fragment of the viral genome; 2. the *detection* system that is based on the precipitation of an insoluble product at those sites of the microarray where the hybridization of the amplified products by specific probes takes place. During RT-PCR, amplified products are labelled with biotin. After amplification, they hybridize with their respective specific probes that are immobilized on the array and then incubated with a streptavidin-peroxidase conjugate. The conjugate binds via streptavidin with the biotin present in the amplified

products (which are also bound to their specific probes). While in the presence of o-dianisidine, the peroxidase activity of the conjugate induces the appearance of an insoluble product which precipitates at the hybridization sites of the microarray.

Viruses analyzed include: adenovirus; bocavirus; coronavirus; enterovirus (echovirus); influenza virus A (subtypes H3N2 human, H1N1 human, B, C and H1N1/2009); metapneumovirus (subtypes A and B); parainfluenza virus 1, 2, 3, and 4 (subtypes A y B); rhinovirus; respiratory syncytial virus type A (VSR-A); respiratory syncytial virus type B (VSR-B).

#### **Diagnostic definitions:**

Probable pneumonia: at least a positive sputum culture.

Definite pneumonia:

1. positive blood culture
2. positive cultures of pleural effusion fluid or aspiration transthoracic puncture
3. elevated serum levels of IgM against *C. pneumoniae* ( $\geq 1:64$ ), *C. burnetii* ( $\geq 1:80$ ), and *M. pneumoniae* (any positive titre) were found at first serological test or in case of a fourfold rise in titers for any of them at 4-6 weeks.
4. Positive urinary Antigens for *L. pneumophila* or *S. pneumoniae*;
5. TBAS  $>10^5$  ufc/ml, BAL  $>10^4$  ufc/ml;
6. *Aspergillus spp.* detection along with radiographic (Computerised Tomography) or histological evidence.
7. positive micro arrays results

*Not pneumonia:* in case of diagnosis of mycobacterial infection, cancer, pulmonary embolism, etc.



**Table E1. Modified Charlson Index**

Myocardial infarction .....	1
Congestive heart failure.....	1
Peripheral vascular disease.....	1
Cerebrovascular disease.....	1
Dementia .....	1
Hemiplegia .....	2
Chronic pulmonary disease.....	1
Connective tissue disease.....	1
Ulcer disease.....	1
Liver disease mild .....	1
Liver disease moderate/severe.....	3
Diabetes mellitus without end organ damage.....	1
Diabetes mellitus with end organ damage.....	2
Chronic renal failure.....	2

Note: Modification include exclusion of condition of immunosuppression

**Table E2. Barthel scale**

<p><b><u>Bowels</u></b>          0 = incontinent (or needs to be given enemas / suppositories)          1 = occasional accident (rare; &lt; once a week)          2 = continent (If needs enema/supp. Must manage himself)</p>	<p><b><u>Transfers (bed to chair and back)</u></b>          0 = unable, no sitting balance, needs hoist or complete lift by 2 persons          1 = major help (one or two people, physical), can sit          2 = minor help (verbal or minor physical E.g. Help from spouse)          3 = independent (no help; includes locking of wheelchair if necessary)</p>
<p><b><u>Bladder</u></b>          0 = incontinent, or catheterized and unable to manage alone          1 = occasional accident (max. once in 24 hrs. needs help with device)          2 = continent (able to use any device E.g. Catheter if necessary). Continent for more than 7 days.</p>	<p><b><u>Mobility (on level surfaces)</u></b>          0 = immobile including being wheeled by another          1 = wheelchair independent, including corners          2 = walks with help of one person (verbal or physical) including help up into walking frame or other help standing.          3 = independent (but may use any aid except rotator; for example, stick or frame) &gt; 50 m / about house.</p>
<p><b><u>Grooming</u></b>          0 = needs help with personal care          1 = independent (must be able to do all personal activities E.g. washing hands and face/ combing hair/ cleaning teeth/shaving, implements provided)</p>	<p><b><u>Dressing</u></b>          0 = dependent (needs help)          1 = needs help but can do about half unaided          2 = independent (including buttons, zips, laces, etc.)</p>
<p><b><u>Toilet Use</u></b>          0 = dependent (unable to manage without major assistance)          1 = needs some help (able to manage with minor help balancing, handling clothes or toilet paper. Still able to use toilet)          2 = independent (can get on and off alone, able to handle clothes, wipe self, flush toilet, empty commode completely unaided)</p>	<p><b><u>Stairs</u></b>          0 = unable (needs lift or cannot negotiate stairs)          1 = needs help (verbal or physical supervision, carrying aid)          2 = independent (must carry walking aid if used)</p>
<p><b><u>Feeding</u></b>          0 = unable (needs to be fed)          1 = needs help cutting, spreading butter, but can feed self          2 = independent (able to use any necessary device in a reasonable time, able to cut up food, use condiments, spread butter etc. on his own, food may be placed within reach)</p>	<p><b><u>Bathing</u></b>          0 = dependent (needs help)          1 = independent (able to wash self all over, may be in shower. A full bath or standing and sponging all over. Includes getting into and out of bath or shower)</p>

**Total = 0-100 points (0-90 is using a wheeling seat)**

**Table E3. Dysphagia scale**

- Degree 0: absent
- Degree 1: solid food dysphagia
- Degree 2: bland solid dysphagia
- Degree 3: liquids dysphagia
- Degree 4: total dysphagia (incapability to swallow saliva)

**Table E4. Positive microbiological results in HCAP and CAP patients**

	Cases		Controls		P value
<b>Blood culture (days 0-1)</b>	24/158	15.2%	18/177	10.2%	<b>0.223</b>
<b>Gram staining</b>	14/62	22.6%	13/77	16.9%	<b>0.529</b>
<b>Quantitative culture (days 0-5)</b>	53/233	22.7%	63/229	29.7%	<b>0.237</b>
- sputum	44	91.7%	58	92.1%	<b>0.783</b>
- BAS	6	12.5%	6	9.5%	<b>0.848</b>
- pleural liquid	2	4.2%	3	4.8%	<b>0.755</b>
- BAL	1	2.1%	1	1.6%	<b>0.599</b>
<b>Urinary Ag <i>Legionella pneumophila</i></b>	5/198	2.5%	8/215	3.7%	<b>0.679</b>
<b>Urinary Ag <i>S. pneumoniae</i></b>	47/194	24.2%	46/217	21.2%	<b>0.539</b>
<b>MRSA swab (days 0-5)</b>	19/122	15.6%	7/124	5.6%	<b>0.020</b>
<b>Viral swab (day 0)</b>	38/146	26.0%	39/147	26.5%	<b>0.972</b>
<b>Serology tests for atypical pathogens(day 0)</b>	8/164	4.8%	6/175	3.4%	<b>0.691</b>

Note: fractions indicate the number of positive results out of the number of performed tests. Viral swab investigates the following viruses: adenovirus; bocavirus; coronavirus; enterovirus (echovirus); influenza virus A (subtypes H3N2 human, H1N1 human, B, C and H1N1/2009); metapneumovirus (subtypes A and B); parainfluenza virus 1, 2, 3, and 4 (subtypes A y B); rhinovirus; respiratory syncytial virus type A (VSR-A); respiratory syncytial virus type B (VSR-B). Atypical pathogens: *Mycoplasma pneumoniae*, *Coxiella burnetii*, *Chlamydia pneumophila*).

Abbreviations. BAS: bronchial aspirate; BAL: bronchio-alveolar lavage; MRSA: methicillin resistant *Staphylococcus Aureus*.

**Table E5. Viral pathogens identified in HCAP and CAP populations.**

	HCAP		CAP	
	N	%	N	%
Syncytial respiratory virus	7	5%	1	1%
Rhinovirus	5	3%	2	2%
Adenovirus	2	1%	3	2%
Influenzavirus A-B	4	2%	3	3%
Parainfluenza viruses 1-3	6	4%	1	1%
Metapneumovirus A-B	1	1%	1	1%
Enterovirus	1	1%	0	0%
Total number	26	17%	11	9%

**Table E6. Microbial combinations among patients with pneumonia of mixed aetiology (two or more pathogens).**

	<b>Cases</b>	<b>Controls</b>
<i>S. pneumoniae</i> plus virus	2	4
<i>S. pneumoniae</i> plus <i>Legionella pneumophila</i>	3	3
<i>S. pneumoniae</i> plus other bacteria	3	0
<i>S. pneumoniae</i> plus <i>Pseudomonas aeruginosa</i>	0	1
<i>S. pneumoniae</i> plus <i>E. coli</i>	0	1
<i>Staphylococcus aureus</i> plus virus	1	0
<i>Pseudomonas aeruginosa</i> plus virus	1	0
Two viruses	1	1

Note. Other bacteria: *Moraxella catarrhalis*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*.

**Table E7. Significant univariate and multivariate logistic regression analyses of predictors of 1-month mortality.**

Variable	Univariate		Multivariate*	
	p-value	OR	95% CI	p-value
<b>Age (+5 years)</b>	<b>0.016</b>	-	-	-
<b>Tobacco</b>	<b>0.078</b>	-	-	-
<b>Alcohol</b>	<b>0.068</b>	-	-	-
<b>Dysphagia</b>	<b>0.003</b>	-	-	-
<b>HCAP</b>	<b>0.011</b>	-	-	-
Admission to Respiratory Disease Department	<b>0.132</b>	-	-	-
Admission to Internal Medicine Department	<b>0.054</b>	-	-	-
<b>Chronic cardiac disease</b>	<b>0.009</b>	<b>9.95</b>	<b>2.84-34.94</b>	<b>&lt;0.01</b>
<b>Neurological disease</b>	<b>0.028</b>	-	-	-



<b>Swallowing disorders</b>	<b>0.005</b>	-	-	-
<b>Coronary heart disease</b>	<b>0.001</b>	-	-	-
<b>Cardiac failure</b>	<b>0.234</b>	-	-	-
<b>Cerebrovascular disease</b>	<b>0.007</b>	-	-	-
<b>Dementia</b>	<b>0.001</b>	-	-	-
<b>Hemiplegia</b>	<b>0.149</b>	-	-	-
<b>Diabetes mellitus with end organ damage</b>	<b>0.019</b>	-	-	-
<b>Chills</b>	<b>0.047</b>	-	-	-
<b>Pleuritic pain</b>	<b>0.043</b>	-	-	-
<b>Cough</b>	<b>0.001</b>	-	-	-
<b>Expectoration</b>	<b>0.001</b>	-	-	-
<b>Altered mental status</b>	<b>0.002</b>	-	-	-
<b>Crackles</b>	<b>0.005</b>	-	-	-
<b>Rhonchus</b>	<b>0.020</b>	-	-	-
<b>Labial Herpes</b>	<b>0.000</b>	-	-	-
<b>Alveolar infiltrate</b>	<b>0.006</b>	<b>0.16</b>	<b>0.35-0.69</b>	<b>0.01</b>
<b>CRB-65</b>	<b>0.005</b>	-	-	-
<b>Length of hospital stay</b>	<b>0.026</b>	<b>1.10</b>	<b>1.04-1.16</b>	<b>&lt;0.01</b>
Length of stay in Emergency department (hours)	<b>0.239</b>	-	-	-
<b>Barthel index</b>	<b>0.000</b>	<b>0.97</b>	<b>0.96-0.98</b>	<b>&lt;0.01</b>
Respiratory rate>30 bpm	<b>0.002</b>	<b>1.16</b>	<b>1.072-1.25</b>	<b>&lt;0.01</b>

Footnote: For univariate analysis variables with p<0.25 were considered significant for posterior multivariate analysis.

**Table E8. Significant univariate and multivariate logistic regression analyses of predictors of 1-year mortality.**

<b>Variable</b>	<b>Univariate</b>		<b>Multivariate*</b>	
	<b>p-value</b>	<b>OR</b>	<b>95% CI</b>	<b>p-value</b>
Age (+5 years)	<b>0.12</b>	<b>1.29</b>	<b>1.07-1.55</b>	<b>&lt;0.01</b>
HCAP	<b>&lt;0.01</b>	<b>3.15</b>	<b>1.63-6.09</b>	<b>&lt;0.01</b>
Days of ICU stay	<b>&lt;0.01</b>	<b>1.26</b>	<b>1.09-1.45</b>	<b>&lt;0.01</b>
Admission to Respiratory Disease Department	<b>0.02</b>	-	-	-

Admission to Internal Medicine Department	<b>0.32</b>	-	-	-
Admission to Geriatric Department	<b>0.10</b>	-	-	-
Neurological disease	<b>0.45</b>	-	-	-
COPD	<b>0.36</b>	-	-	-
Interstitial lung disease	<b>0.44</b>	-	-	-
Asthma	<b>0.268</b>	-	-	-
Cerebrovascular disease	<b>&lt;0.01</b>	-	-	-
Dementia	<b>&lt;0.01</b>	-	-	-
Hemiplegia	<b>&lt;0.01</b>	-	-	-
Chills	<b>&lt;0.01</b>	-	-	-
Pleuritic pain	<b>0.03</b>	<b>0.40</b>	<b>0.16-0.99</b>	<b>0.05</b>
Cough	<b>&lt;0.01</b>	-	-	-
Expectoration	<b>0.06</b>	-	-	-
Altered mental status	<b>&lt;0.01</b>	-	-	-
Wheezing	<b>0.08</b>	-	-	-
Rhonchus	<b>0.04</b>	-	-	-
Labial Herpes	<b>&lt;0.01</b>	-	-	-
CRB-65	<b>&lt;0.01</b>	-	-	-
Length of hospital stay (days)	<b>&lt;0.01</b>	-	-	-
Length of stay in Emergency department (hours)	<b>0.03</b>	-	-	-
Barthel index	<b>&lt;0.01</b>	-	-	-
Respiratory rate>30 bpm	<b>&lt;0.01</b>	<b>2.16</b>	<b>1.04-4.48</b>	<b>0.04</b>

Footnote: For univariate analysis variables with p<0.25 were considered significant for posterior multivariate analysis.

Figure E1. Patients' distribution according to Pneumonia Severity Index (PSI)

